# THE DEVELOPMENT OF PHYSIOLOGICAL CHEMISTRY

## IN THE UNITED STATES


BY

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# GENERAL INTRODUCTION

## American Chemical Society Series of Scientific and Technologic Monographs

By arrangement with the Interallied Conference of Pure and Applied Chemistry, which met in London and Brussels in July, 1919, the American Chemical Society was to undertake the production and publication of Scientific and Technologic Monographs on chemical subjects. At the same time it was agreed that the National Research Council, in coöperation with the American Chemical Society and the American Physical Society, should undertake the production and publication of Critical Tables of Chemical and Physical Constants. The American Chemical Society and the National Research Council mutually agreed to care for these two fields of chemical development. The American Chemical Society named as Trustees, to make the necessary arrangements for the publication of the monographs, Charles L. Parsons, Secretary of the American Chemical Society, Washington, D. C.; John E. Teeple, Treasurer of the American Chemical Society, New York City; and Professor Gellert Alleman of Swarthmore College. The Trustees have arranged for the publication of the American Chemical Society series of (a) Scientific and (b) Technologic Monographs by the Chemical Catalog Company of New York City.

The Council, acting through the Committee on National Policy of the American Chemical Society, appointed the editors, named at the close of this introduction, to have charge of securing authors, and of considering critically the manuscripts prepared. The editors of each series will endeavor to select topics which are of current interest and authors who are recognized as authorities in their respective fields. The list of monographs thus far secured appears in the publisher's own announcement elsewhere in this volume.

The development of knowledge in all branches of science, and especially in chemistry, has been so rapid during the last fifty years and the fields covered by this development have been so varied that it is difficult for any individual to keep in touch with the progress in branches of science outside his own specialty. In spite of the facilities for the examination of the literature given by Chemical Abstracts and such compendia as Beilstein's Handbuch der Organischen Chemie, Richter's Lexikon, Ostwald's Lehrbuch der Allgemeinen Chemie, Abegg's and Gmelin-Kraut's Handbuch der Anorganischen Chemie and the English and French Dictionaries of Chemistry, it often takes a great deal of time to coördinate the knowledge available upon a single topic. Consequently when men who have spent years in the study of important subjects are willing to coördinate their knowledge and present it in concise, readable form, they perform a service of the highest value to their fellow chemists.

It was with a clear recognition of the usefulness of reviews of this character that a Committee of the American Chemical Society recommended the publication of the two series of monographs under the auspices of the Society.

Two rather distinct purposes are to be served by these monographs. The first purpose, whose fulfilment will probably render to chemists in general the most important service, is to present the knowledge available upon the chosen topic in a readable form, intelligible to those whose activities may be along a wholly different line. Many chemists fail to realize how closely their investigations may be connected with other work which on the surface appears far afield from their own. These monographs will enable such men to form closer contact with the work of chemists in other lines of research. The second purpose is to promote research in the branch of science covered by the monograph, by furnishing a well digested survey of the progress already made in that field and by pointing out directions in which investigation needs to be extended. To facilitate the attainment of this purpose, it is intended to include extended references to the literature, which will enable anyone interested to follow up the subject in more detail. If the literature is so voluminous that a complete bibliography is impracticable, a critical selection will be made of those papers which are most important.

The publication of these books marks a distinct departure in the policy of the American Chemical Society inasmuch as it is a serious attempt to found an American chemical literature without primary regard to commercial considerations. The success of the venture will depend in large part upon the measure of coöperation which can be secured in the preparation of books dealing adequately with topics of general interest; it is earnestly hoped, therefore, that every member of the various organizations in the chemical and allied industries will recognize the importance of the enterprise and take sufficient interest to justify it.

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# PREFACE

In the following pages the attempt has been made to show by explicit statements of actual experimental accomplishments, at different times and in different places, by many workers and groups of workers, something of the progress which has been made in this country during the past half century in the field of physiological chemistry. The story could not be told at all adequately, with that completeness which might well seem desirable and with due credit to all those who have contributed to the upbuilding of the science, in the limited number of pages available. Selection necessarily had to be made and judgment exercised, not alone on the basis of scientific excellence, physiological value, or relative merit, but also with a view to the recognition of the numerous centers of learning throughout the country, both large and small, where physiological chemistry or biochemistry had gained a foothold.

Critical analysis of the accomplishments recorded here has not been attempted; care has been taken to avoid playing the part of a critic of either work or workers. Neither is this an appraisal of the extent of biochemical knowledge in any of the fields of investigation considered, but rather an exposition of the growth and development of physiological chemistry in this country, as indicated by the character of the work undertaken and by the rapid

increase in numbers of those occupied in research, as well as by the wide-spread increase of laboratories for instruction and research.

From the earlier chapters it is hoped that times long forgotten may be reconstructed by many who are still active workers, and that by the younger generation there may be gleaned some conception of the conditions that prevailed at a time when the biological sciences based on experimentation were just beginning to gain recognition in this country, long before physiological chemistry had come of age. From these pages in their entirety, it may be possible to trace out in some degree the sequence of discovery, to gain some insight into the tendencies of physiological thought with perhaps a clearer understanding of the relations of yesterday, today and tomorrow. The work of yesterday is accomplished, but who can tell how far the influences of that work will extend into the future? Success and failure both pave the way for later progress, and it requires little or no imagination to realize that each forward step, no matter how weak and halting, is an aid in carrying forward an experimental science to a higher stage of development.

The accomplishments of yesterday present a suggestive outlook for the achievements of tomorrow, but the gift of accurate prophecy is denied to most of us. Still, taking the events of the past fifty years, and giving due weight to the steadily mounting interest in physiological chemistry throughout the country, to the phenomenal growth of laboratories devoted to the subject and the great increase in well-trained workers, it does not seem at all unwarranted to express the opinion that the tomorrow of

physiological chemistry in the United States will witness a great forward advance replete with benefits for the science of physiology, as a study of function, in the broadest sense of the term.

Finally, I must add that I am indebted to Professor Lafayette B. Mendel for his kindness in reading portions of these pages in manuscript and for various suggestions made.

Russell H. Chittenden

New Haven, Connecticut,
June, 1930.

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# CHAPTER I

Standing of physiological chemistry and physiology in this country in 1870-1880—Position of physiological chemistry in France and Germany during the same period—Necessity for the American student to go to Germany for training in physiological chemistry—Experience of the writer at Strassburg and Heidelberg in 1878-1879.

In 1878, as a young student especially interested in the applications of chemistry to physiology, I went to Germany for the knowledge and experience that could not be acquired in the United States. At that date, physiological chemistry had no real standing in this country, nor indeed in England; not enough at least to warrant a laboratory, worthy of the name, for either instruction or research. The experimental method was hardly recognized in the study of physiology, and even in England it was not until 1874 that Sir Michael Foster, then a young instructor at University College, London, attempted "practical *instruction in physiology, histology and rudimentary* physiological chemistry," stated by Foster to be the "beginning of the teaching of practical physiology in England."

In this country conditions were essentially the same. When Henry P. Bowditch returned to America in 1871, after several years of work in Germany, especially in the physiological laboratory of Carl Ludwig at Leipzig, there were no suitable quarters for him to carry on experimental

work in physiology at the Harvard Medical School. He was the newly appointed assistant professor of physiology at Harvard, but at that date a laboratory was not considered an essential. Bowditch, however, had brought back with him from Leipzig considerable apparatus for experimental purposes, and for his accommodation two small rooms in the attic of the Medical School building were fitted up, where he and a few advanced students and instructors were able to carry on experimental work. Thus, was established "the first physiological laboratory for the use of students in the United States."[1]

The teaching of chemistry by rational methods was much more advanced in this country in 1870 than the teaching of physiology. Opportunities for advanced work and for research in well-appointed chemical laboratories under the guidance of competent instructors were by no means rare in America at that date, but for physiology there were no such opportunities and indeed very little demand for them. Physiology was simply a part of a rather crude medical curriculum, in which frequently it joined hands with anatomy and the two together were taught by the same instructor, or as a part of the "Institutes of Medicine" it was intermingled with general pathology and the elements of clinical medicine. Specialized instruction in the so-called medical sciences was not even thought of and physiology in particular had no standing at all outside the medical curriculum.

How different today, after fifty years in the use of the experimental method! In the words of the president of

[1] "Biographical Memoir of Henry Pickering Bowditch," by W. B. Cannon, *Memoirs of the National Academy of Sciences* XVII: 186.

the section on Physiology of the British Association for the Advancement of Science,[2] "Physiology has a threefold appeal—as the master-key of medicine its practical value is self-evident, as a science it has now a distinctive position, while its relations to philosophy command the attention of all thoughtful men." Again, "physiology is something more than biochemistry and biophysics; it is and will always remain a biological subject."

In view of the situation in America in 1870-1880, physiological chemistry might well wonder where it belonged, what indeed its very name stood for. As an experimental science it must of necessity have a place, if not in the sun, at least where it could develop unhampered. Obviously, its apparatus and equipment in general must be largely chemical, but the mind that was to direct and use the tools must have a sound physiological training. Otherwise there could be no accurate application of chemical facts to physiological teaching or to the proper interpretation of physiological phenomena.

There was, however, at the above date no true appreciation of the important part chemistry might play in the expanding of knowledge of the functions of the animal body, and consequently so-called physiological chemistry was hardly more than a name, and that of uncertain significance. For many years it stood on the border line between chemistry and physiology and in this country at least there were many arguments, heard even as late as 1900, as to whether physiological chemistry should be under the jurisdiction of the department of chemistry or of physiology in our universities.

[2] Professor Charles A. L. Evans, *Science*, **68**: Nr. 1760 (1928).

For years the American medical school presented "medical chemistry" as the sole approach to the chemical aspects of physiology. In those days the aid of chemistry was invoked mainly in the determination of the composition of animal and vegetable tissues and fluids, together with some testing for abnormal or pathological constituents. Experimental physiology so far as it had recognition in this country, dealt mainly with muscles and nerves, heart and respiration; the experimental method was limited to the physics of physiology, and the possibilities of chemical physiology were either unrecognized or ignored as of little practical importance.

All this is not so strange as it may seem in the light of present-day developments, for even in Germany, where physiology and physiological chemistry had been greatly advanced, there was a certain ambiguity in the position held by the latter branch of study. This is perhaps indicated by the diversity of titles in German publications dealing with physiological chemistry. Thus in the German edition of Berzelius' *Lehrbuch der Chemie,* translated by Friedrich Wöhler and issued in 1840, the ninth volume was entitled *Thier-Chemie* and covered such knowledge as then existed regarding the chemistry of tissues and the processes of the body. In 1846 appeared Liebig's work *Die Thierchemie oder die Organische Chemie in ihrer Anwendung auf Physiologie und Pathologie.* In 1866-1871, Hoppe-Seyler, while at Tübingen, issued his *Medicinisch-Chemische Untersuchungen,* and in 1871 Gorup-Besanez's *Anleitung Zur Qualitativen und Quantitativen Zoochemischen Analyse* was published. In 1873, Richard Maly of the University of Innsbruck began the

publication of his celebrated *Jahresbericht über die Fortschritte der Thierchemie*, containing a report of all experimental work in physiological and pathological chemistry for the year.

It is significant that Dr. Maly's title was "professor of applied medical chemistry" in the university, and that the title of the year book was progress in "animal chemistry," these two titles being indicative in a way of the somewhat anomalous position of physiological chemistry at that date. The situation is further complicated by the fact that many years earlier various books had been written bearing the distinctive title of physiological chemistry. Thus, in 1826, there was published in Leipzig by Friedrich Ludwig Hünefeld of Breslau *Physiologische Chemie des Menschlichen Organismus zur Beförderung der Physiologie und Medicin.* In 1844-1851 appeared Mulder's *Allgemeine Physiologische Chemie* and in 1844 Marchand of the University of Halle issued his *Lehrbuch der Physiologischen Chemie.*

These examples will suffice to indicate what is, I think, quite apparent, that at the above dates there was a disposition to look on what we now term physiological chemistry, or biological chemistry, as simply a branch of chemistry in which the chemical composition, and later chemical constitution, of the many substances entering into the make-up of animal tissues and fluids represented the main subjects to be studied. Such a position was natural enough at that time, especially since the rapid development of organic chemistry was opening up new views of chemical relationship of great significance in the study of metabolic changes. Further, vision was limited

because of the wide-spread disposition to view physiology *mainly* as a study of functions explainable solely by physical methods.

There was so much to learn, especially so much that was directly helpful to medicine, that physical physiology naturally became dominant and usurped all the rights of a broader conception of physiology as a study of animal functions. In fact, for many years, the study of muscle-nerve physiology practically eclipsed all other branches of experimental work in physiology. Consequently, chemical physiology had little or no recognition as such, and animal chemistry became a sort of appendix, useful to physiology to be sure, but not an integral part of the developing science. Animal chemistry was in fact looked on by many at the above dates as bearing much the same relationship to physiology as anatomy did. The latter had to do with anatomical structure, while physiological chemistry had to do with the chemical structure of the animal body. The survival of this conception is seen in Sir Michael Foster's *Textbook of Physiology* as published in 1893, in which there is an appendix by Sheridan Lea, entitled *The Chemical Basis of the Animal Body.*

Again, physical physiology was contributing so much of value to scientific medicine that experimental work along these lines was gradually assuming a relation to medicine that tended to overemphasize physiology as a branch of medicine. No one would question for a moment the importance of a close association of physiology with medicine, but the medical man of 1870 or thereabouts was slow to realize the opportunity which chemistry

offered for expanding knowledge of physiological processes. As he saw it, so-called physiological chemistry presented little of real physiological importance, nothing which could compare with the helpfulness of the new findings in physical physiology; so he was disposed to relegate chemical studies to a compartment by themselves, and more inclined to refer to them under the term of medical chemistry than to recognize them as an essential part of any broad scheme of physiological studies.

There was lacking a just appreciation of the importance of encouraging physiological inquiry along broad scientific lines without regard to any specific application, medical or otherwise, and without restriction to any given method of experimentation, whether physical or chemical. Physiology has to do with the recognition and interpretation of the fundamental phenomena of life and as we know today the chemical processes normally taking place in the animal body are fully as important as those usually classified under the head of physical physiology, but in 1870 and for some years later, in this country at least, physiological chemistry was set apart and its pedigree somewhat uncertain.

In 1870-1880, France and Germany stood forth as the two countries where the study of experimental physiology was being vigorously prosecuted. Claude Bernard of Paris and Carl Ludwig of Leipzig were the two men above all others of that period who are to be given the credit of advancing experimental physiology to a level equal to that occupied by the other experimental sciences. Prior to their time, animal experimentation was practically

unrecognized, indeed was frowned upon, considered as worse than useless and its advocates derided as futile workers, whose efforts could lead to no useful results. But Claude Bernard, with the intellectual inheritance of Lavoisier, with a broad interest in physiology, and with a brilliancy that attracted and held the attention of the scientific world, made so many striking discoveries that under his leadership experimental physiology was raised to a level in France it had never before occupied.

Claude Bernard died in 1878, but what he had accomplished endured long after him and furnished an example of the fruitfulness of research in both the physical and chemical sides of physiology. The discovery of hepatic glycogen and its relation to blood sugar in health and disease, his discoveries regarding the digestive power of the pancreatic juice, together with his many researches in the domain of physical physiology all testify to his breadth of view as a physiologist and to his equal interest in the two branches of the science.

Carl Ludwig was appointed professor of physiology at the University of Leipzig in 1865. He was an experimenter of the highest rank who created a school of experimental physiology that drew students from all over the world. His interests lay primarily in the field of physical physiology, but Germany in 1870-1880 was still feeling the influence of von Liebig's classical work in chemistry, and physiological chemistry had due recognition in the Leipzig laboratory through a more or less independent department under the jurisdiction of the distinguished chemist, Professor E. Drechsel, whose scientific work for many years brought added renown to the

Leipzig laboratory and at the same time added greatly to existing knowledge in the field of physiological chemistry.

But Leipzig was by no means the only university center in Germany where experimental work in physiology and physiological chemistry was being vigorously prosecuted. In 1878, that country was without question the Mecca toward which American and English students anxious to pursue experimental work in physiology—and indeed in most of the biological sciences—naturally turned for guidance and inspiration. There, were to be found laboratories and more important still men of genius whose lives were devoted to experimental work in physiology and whose efforts were opening up new chapters in the study of function and expanding old ones. Further, what was of supreme importance, chemical physiology in all its varied aspects, was being given equal consideration with physical physiology. Each laboratory, to be sure, had its own atmosphere, dependent in large measure upon the type of work the master was most interested in, and there was great variety of choice. The men at the head of these university laboratories bore names that are today household words in the history of physiological science.

In Berlin, Emil DuBois-Reymond, professor of physiology at the University, was an outstanding figure, whose researches in animal electricity and on the functions of nerves, had gained him wide recognition. Associated with him, but in a separate laboratory, was Baumann, whose work lay wholly in the field of chemical physiology. Also at Berlin was E. Salkowski, professor in the University, and head of the chemical laboratory of the Pathological

*Institute of Berlin, whose time was given entirely to the study of problems in physiological and pathological chemistry.*

At Breslau, R. Heidenhain was the professor of physiology and in his laboratory a large amount of experimental work was being carried on, especially in the study of digestion and the changes taking place in secreting cells during the various stages of activity. Here, too, was an allied department of physiological chemistry presided over by the distinguished colleague Röhmann.

In Bonn, E. F. W. Pflüger was the professor of physiology, a man whose successful experimental work in both physical physiology and physiological chemistry had given him a well-deserved reputation for breadth of physiological knowledge. From his laboratory came a wealth of new material that contributed much to a better understanding of physiology.

At Munich, Pettenkofer and Voit were outstanding figures from their work on respiration with the calorimeter, while the younger man, Carl von Voit, became the most renowned authority in the field of human nutrition, in whose laboratory were trained many of the men, German, American and English, who later became authorities in this branch of physiology.

In Strassburg was Felix Hoppe-Seyler, professor of physiology, the recognized head of an "Institute of Physiological Chemistry," the only separate foundation of its kind in Europe at that date. In this Institute a large number of the leaders in physiological chemistry began their labors, notably, Kossel, Baumann, Hofmeister and others.

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At Tübingen, G. Hüfner was the professor of physiology, whose work on the chemistry of blood had made him a recognized authority in this field of physiology. At the University of Heidelberg Willy Kühne was the professor of physiology, a man who had gained distinction both as a worker in muscle and nerve physiology and as an investigator in the field of chemical physiology, his *Lehrbuch der Physiologischen Chemie* (1868) having enjoyed broad recognition.

These statements will suffice to indicate the wealth of opportunity for experimental work at the university centers of Germany in 1878. It was a time when activity in the field of chemical physiology had become very pronounced. Recognition of the importance of the chemical aspects of physiology was growing each year and workers in this field of research were increasing rapidly in numbers. Physiological chemistry was beginning to create for itself a distinct position, indicated not alone by the number of the contributions it was making to existing knowledge but even more by the character of these contributions.

It is easy to judge the growth of a given branch of science by noting the facilities for publication at the disposal of the workers in that particular field. Prior to 1873, there was no distinctive journal for the publication of the results of work in physiological chemistry. Papers appeared in all kinds of journals, both chemical and physiological as well as in more strictly medical journals. The result was unfortunate. Good work was often buried and forgotten, and the research worker was greatly handicapped by his inability to find easily the record of work

*done by others in his field. The point to be stressed, however, is that prior to 1873, there was not sufficient output of research work in physiological chemistry to compel the* creation of special journals or other publications.

Thus, in Germany, articles on physiological chemistry were to be found in Du Bois-Reymond's *Archiv für Physiologie,* in the *Archiv für die gesammte Physiologie,* in the *Zeitschrift für Biologie,* in the *Centralblatt für die Medicinische Wissenschaften,* in Liebig's *Annalen der Chemie und Pharmacie,* in the *Journal für Praktische Chemie,* in the *Berichte der Deutschen Chemischen Gesellschaft,* to mention only the more conspicuous publications. But in 1873, as previously noted, there appeared the first volume of the *Jahresbericht über die Fortschritte der Thierchemie,* in which was collected brief summaries of all the work published in physiological chemistry for the year 1871. Thus, was started a publication, which is emphasized here simply to mark a conspicuous birthday in the life history of physiological chemistry. More significant still was the establishment in 1877 of the *Zeitschrift für Physiologische Chemie* by Hoppe-Seyler and a small group of co-workers in this field, thus creating a distinctive publication, devoted solely to the needs of research workers in the field of chemical physiology; convincing evidence of the increasing activity in this branch of physiological science.

Today, after a period of fifty years, when America is in a sense pre-eminent in the field of physiological chemistry, with adequately equipped laboratories devoted to this science in most of her universities, with research institutions heavily endowed, with professors and research

workers well trained for their task and with numerous *facilities for publication, it is well* to remember the years *of effort the physiologists and chemists of Germany gave* to the development of physiological chemistry. Every student of physiology at all interested in the development of his science is familiar with the names and accomplishments of those early workers who wrought so successfully in molding physiological opinion. Through their efforts physiological chemistry gained added dignity and a more definite position as an integral part of physiology.

In this work, France likewise participated and the names of many French physiologists might well be added as kindred contributors to the growth of a science which is doing so much to explain many of the processes of life. It must be remembered that these early pioneers were zealous workers in a field of research that had scanty recognition in this country and likewise in England. To them must be given the honor of having started, and continued for many years, the movement which has resulted in the present-day expansion of knowledge in that branch of science which we term physiological chemistry; an expansion of knowledge that has altered in many ways our conceptions of physiology as a study of function.

As already indicated, in 1878 any American student desirous of making progress in physiological chemistry had no recourse other than going to Germany for the knowledge and experience he needed to help him on his way. He might be well trained in chemistry, according to the standards of that date, but he would be wholly lacking in the knowledge of methods, in the proper way of ap-

proach, for the study of physiological problems. He required training in the experimental methods so essential for the successful prosecution of a physiological inquiry. And above all he must acquire a proper perspective, to be gained only by careful observation and experience, under the guidance of a master mind. When I reached Germany in the fall of 1878, the University of Heidelberg was finally selected as the place best suited to my needs.

Professor Kühne had been trained under Virchow of Berlin, Carl Ludwig of Leipzig and Claude Bernard of Paris, three masters of pre-eminent ability. He had succeeded Helmholtz as professor of physiology at Heidelberg, when the former was called to Berlin, but unlike the latter he was more interested in muscle-protoplasm, in muscle and nerve physiology, nerve endings, and the chemico-physiological processes of digestion than in the sense organs and their functional activity. He it was who following in the footsteps of Bernard worked out the enzyme trypsin of the pancreatic juice, and threw great light upon the primary products of gastric and pancreatic proteolysis. He had worked with Bernard on glycogenic functions and consequently anything relating to glycogen had for him special interest, a fact from which I was to profit later. Kühne was obviously a man broadly trained in the field of physiology and his leanings toward the chemical side of physiology seemed to promise a helpful atmosphere for one who was ambitious to acquire knowledge and experience in physiological chemistry.

Possibly, it may not be amiss to introduce a brief explanation of the circumstances which led finally to the

selection of Heidelberg as the place of study, and a word or two regarding the somewhat peculiar conditions under which entrance to the laboratory was attained, for fifty years ago the privileges of a famous German research laboratory were not open to every applicant who appeared. When I arrived at Heidelberg I had nothing with me in the way of credentials aside from my visiting card, a somewhat awkward situation for a young and unknown American seeking to gain admission to the laboratory of a distinguished German scholar. This situation had arisen owing to the fact that before leaving New Haven all necessary arrangements had been made for entrance to the laboratory of Hoppe-Seyler at Strassburg, and all letters of introduction and other credentials were addressed to the authorities of that University. On reaching Strassburg, however, and presenting the letters of introduction, and after viewing the facilities provided in the laboratories, there arose a grave suspicion that perhaps Strassburg after all was not best adapted for my needs.

There were so many people working in the laboratory, everything was so crowded and lacking in orderly arrangement, there seemed so little opportunity for much personal attention, that there was a natural hesitation to take the decisive step. It must be remembered that the Strassburg of 1878—both city and university—was vastly different from the later years, when Germany had replaced the old and shabby university buildings by artistic and commodious structures abounding with facilities for successful work. There was nothing attractive about either the city or the university in those early years after the Franco-German war of 1870. To be sure, Hoppe-

Seyler was a great man and the laboratory was a beehive of activity, yet it seemed to lack something—atmosphere if you choose—essential for the proper development of a youthful mind that needed guidance and encouragement. Everything had been staked upon this venture abroad, so much depended upon a year or two of successful work and experience, that the risk of failure through unfavorable conditions could not be accepted.

Intuition is not to be wholly ignored, and I went on to Heidelberg with the feeling deep in my heart that the place where such men as Gmelin, Tiedemann, Bunsen, Kirchoff, Helmholtz and Kühne had worked should give inspiration and opportunity, and that there would be found an environment more in harmony with my needs. The situation was somewhat awkward, but assuming a confidence that was not wholly felt, Kühne was sought and the hope expressed that a place might be found in his laboratory. His reception was a gracious one, but it was not difficult to see that the young American, with his imperfect command of German, and with his lack of the customary credentials presented a case quite out of the ordinary, and the outcome seemed dubious. It was explained that all necessary credentials would be forthcoming from America as soon as possible, but that did not seem to interest Kühne particularly. He appeared more interested in the visiting card he held in his hand, and much to the writer's surprise he said, "Are you the Chittenden who published in Liebig's *Annalen* a year or two ago an article on glycogen and glycocoll?"

I was to learn later that Kühne possessed a wonderful memory; anything he had read he rarely forgot, and he

had evidently remembered the somewhat unusual name in connection with a piece of work which dealt with glycogen, to him always a subject of interest. It had happened that on graduating from the Sheffield Scientific School at Yale in 1875 my thesis was on "Glycogen and Glycocoll in the Muscular Tissue of *Pecten irradians.*" This was published in the *American Journal of Science and Arts,* and at the suggestion of Professor S. W. Johnson it was translated into German, with his help, and sent to Liebig's *Annalen der Chemie,* where it was eventually published. Fortunately, Kühne had read this paper and had remembered the name of the author. Going into his library, he came back with the volume of the *Annalen* containing the article and commented on the fact that glycocoll had never before been found free in Nature and that the presence of such large amounts of glycogen in an invertebrate muscle was an interesting and suggestive observation. The atmosphere was completely changed, and my spirits rose accordingly, reaching a still higher level when Kühne remarked that he would find a place for me in the laboratory at once.

Going to Heidelberg was a wise choice, as the experiences of the next few weeks clearly showed, and indeed, the experiences of later years likewise proved. The Heidelberg laboratory was characterized by the great diversity of interests represented, many sides of physiology being studied, both physical and chemical, by the German, English, Russian and American workers there. At the same time, numbers were small, hence there was ample opportunity for Kühne to exercise his influence over us all. He himself carried on his own experimental

work nearby, from which we all derived some profit. After a month in the laboratory, Kühne asked me if I would like to serve as his assistant in the lecture demonstrations. Naturally, this came as a great surprise, but was accepted with alacrity, for while in a sense it was an empty honor, it led to a certain standing in the laboratory and what was of greater importance the very definite advantage of viewing close at hand all operations and experiments, in the carrying out of which Kühne was a master hand.

More than twenty years of close friendly relations with Kühne, up to the time of his death in 1900 only strengthened the first, early impressions of the man. He was as large and broad mentally as he was physically, kind-hearted to a high degree, an ideal teacher and an experimental worker of great skill and daring, whose every thought was given to the advancement of physiology. He was one of that distinguished group of physiologists who helped to make Germany the center of physiological thought during the latter part of the nineteenth century.

## CHAPTER II

Establishment of the first laboratory of physiological chemistry in the United States—Growth of physiological chemistry as a branch of biological study—Beginning of research work in the Sheffield Laboratory of physiological chemistry—Development after 1890—Founding of the American Physiological Society—Establishment of the Journal of Experimental Medicine—Establishment of the American Journal of Physiology—Creation of the American Society of Biological Chemists—Founding of the Journal of Biological Chemistry—Research workers in physiological chemistry.

The first definitive laboratory of physiological chemistry in America for the instruction of students was established in the Sheffield Scientific School at Yale University in 1874 and over this laboratory I was placed in charge. They were very modest quarters at first—a single room capable of accommodating eight students, but fairly well equipped for chemical work—for the movement was an experiment and the authorities obviously felt it unwise to risk much in a venture that might prove unsuccessful.

The creation of this laboratory was the outcome of an earlier movement for the establishment of a course of "Studies Preparatory to Medical Studies" arranged for in 1868 and put in operation the following year. "Thus, was started a course in Biology preparatory to Medicine, one of the first, if not the first, in the country; giving recognition to the principle, not then generally understood, that the study of medicine should rest upon a

foundation of the sciences, particularly those which are now designated as biological. Perhaps, however, equally important was the training such a course of study offered in the scientific method for men who aimed to be proficient in the science and art of medicine."[1] The men primarily responsible for this marked departure from the usual routine of preparation for the study of medicine were Professors Samuel W. Johnson, Daniel C. Eaton, Addison E. Verrill and Daniel C. Gilman.

At first, the course of study consisted of a judicious admixture of mathematics, physics, chemistry including qualitative and quantitative analysis, organic chemistry with some reference to its physiological and medical bearings, botany, zoölogy, comparative anatomy and embryology, together with English, French and German. There was no definitive course in physiological chemistry, but during one term there was placed in the hands of the students a small book entitled *A Manual of Chemical Physiology Including its Points of Contact with Pathology*, by J. L. W. Thudichum, M.D., published in London, 1872, with instructions to work out in the chemical laboratory the various tests and processes contained in the "Analytical Guide" which made up the latter half of the book. It was an impossible procedure for immature students without guidance to derive much profit from, but some knowledge was absorbed and a little experience gained which stimulated the more thoughtful students to further endeavor.

In 1874, however, a well-defined course in physiological

[1] Russell H. Chittenden, "History of the Sheffield Scientific School of Yale University," p. 164.

chemistry was established, designed primarily for the instruction of students intending, later, to enter the study of medicine. Naturally, at this date emphasis was placed mainly on the purely chemical aspects of the subject. This was inevitable, owing partly to the limitations of the instructor and partly to the tendency to consider physiological chemistry simply as a branch of chemistry, dealing with the composition and reactions of substances having physiological significance. As expressed by Professor H. Newell Martin[2] in a review of *Lecture Notes on Chemical Physiology and Pathology,* by Victor C. Vaughan, "It has somehow come to pass that the study of the proximate and ultimate constituents of the dead body, the composition of the secretions of the living body, and some account of the chemistry of digestion, are considered the end and aim of physiological chemistry." Further, the practical value to the physician of a chemical study of food, urine and feces with special reference to the general problem of nutrition was an added factor in determining the character of the instruction during those early years.

Unfortunately, this view of physiological chemistry persisted for years in at least many quarters. Thus as late as 1897 the catalog[3] of one of the foremost medical schools of the country contained the announcement that "instruction in physiological chemistry is given by lectures, recitations and exercises in the laboratory where each student will be taught the chemistry of the carbohydrates, proteids and fats, the chemistry of digestion,

[2] *Am. Chem. J.,* 1: 57 (1879).
[3] Catalog of Harvard University for the college year 1896-1897.

the chemistry and microscopy of the urine and the tests for the important poisons." Not until the year 1898-1899 was there a special instructor in physiological chemistry at Harvard, when Dr. Franz Pfaff served in the double capacity of instructor in pharmacology and physiological chemistry, followed in 1904-1905 by Carl L. Alsberg as assistant in physiological chemistry and in 1905-1906 by Lawrence J. Henderson and Carl L. Alsberg as instructors in biological chemistry.

When I returned to New Haven near the close of 1879, after the experiences in a German laboratory and after intercourse with many of the physiologists of that country, it was inevitable that the character of the work in the laboratory should undergo a decided change. The dynamical aspects of physiological chemistry became the keynote of the instruction. Further, even in those early years the principle that physiological chemistry rightly belonged among the biological sciences; that it was in fact a true biological science, as much so as morphology, zoölogy or botany was becoming firmly fixed. Morphology and physiology were the two main divisions of biology, the one dealing with form and structure, the other with function. Physiological chemistry was to be considered simply as a part of physiology, having to do with the study of the chemical functions of the living organism, animal or vegetable, as the case might be. This being so there was justification for the development of physiological chemistry in a broad biological course of study that aimed to present a more or less complete picture of the phenomena of life. Moreover, the environment so provided tended to emphasize the true position of physi-

ological chemistry as a biological subject not restricted to the necessities of any branch of applied science. To limit the study of physiological chemistry to the needs of medicine, for example, would be to defeat the end in view, *viz.*, the expansion of physiological knowledge in all its varied aspects. Medicine in the end would profit most from a broad development of physiological chemistry, realizing that every new fact brought to light is in time liable to contribute something to that fund of knowledge which is of direct use, and hence of practical value, to the everyday practitioner of medicine.

As the years advanced and physiological chemistry began to justify itself as a branch of biological study, numbers increased rapidly, made up of two classes of students, *viz.*, undergraduates desirous of obtaining a broad scientific foundation as a preparation for the study of medicine, and graduate students who were anxious to undertake advanced and research work in some branch of the subject. It is to be remembered that during these years the medical schools of the country as a rule were still offering only so-called medical chemistry, and that mainly by lectures with demonstrations. Consequently, the broader study of physiological chemistry by experimental methods quickly gained recognition as something unique and full of promise, both as an educational force and as a revealer of truths of value to science and to medicine.

Today, it is easy to look back and see what the applications of chemistry to physiology and to pathology during the past half century have accomplished in the hands of both the scientific worker and the clinician. But fifty years ago, vision was limited and the man of that date who ven-

tured to prophesy that the most important advances in the scientific medicine of the next generation would come from the use of chemical methods of research would have found few followers. Whatever the cause, the laboratory at New Haven soon became an active center for research work, where eventually many men, and women too, were trained to work out problems in physiological chemistry, much to their own educational advancement and in some cases with results of genuine scientific value.

In 1882 at the request of Professor Kühne, I went back to Heidelberg for the summer months to undertake with him an investigation of the primary cleavage products of albuminous bodies, which Kühne had recently discovered. This was the beginning of a period of coöperation in research between the Heidelberg laboratory and the Sheffield laboratory of physiological chemistry which lasted for eight years, with results decidedly stimulating to the scientific atmosphere of the New Haven laboratory. It also resulted quite naturally in focusing the attention of the workers in the laboratory on problems connected with digestion, so that in the Sheffield laboratory many experimental studies were carried on dealing with the chemico-physiological processes of the gastro-intestinal tract.

The experimental work done by Kühne and Chittenden during the eight years was carried on in the two laboratories at Heidelberg and New Haven, all discussions relating to methods and the significance of the findings being by correspondence. The results of the studies were published in the *Zeitschrift für Biologie* under the joint names of Kühne and Chittenden and bore the fol-

lowing titles: *Über die nächsten Spaltungsproducte der Eiweisskörper,* 19, 159-208 (1883); *Über Albumosen,* 20, 11-51 (1884); *Globulin und Globuloses,* 22, 409-422 (1886); *Über die Peptone,* 22, 423-458 (1886); *Myosin und Myosinoses,* 25, 358-367 (1888); *Über das Neurokeratin,* 26, 291-323 (1890). Some of these papers were likewise published in English in the *Transactions of the Connecticut Academy of Arts and Sciences* or in the *American Chemical Journal.* If any excuse is needed for the introduction of the above details, it is to be found in the light they throw on the development in the Sheffield laboratory of interest in protein chemistry and in proteolysis; an interest that was a characteristic of the laboratory and of the experimental work therein for many years, and which was a prelude to the broader experimental work on nutrition which followed.

Research work by graduate students and others in the Sheffield laboratory reached such an amount within a few years that in 1885 a volume of *Studies in Physiological Chemistry*—the first of a series—was issued covering the work accomplished during the college year 1884-1885, a total of eleven papers, most of them having appeared in the *Transactions of the Connecticut Academy,* all the work of Chittenden and his students. While no remarkable discoveries were made, the men who participated in the experimental work covered by these papers brought to light many facts of physiological value which more than justified their efforts and at the same time gave them a training in scientific method and constructive thinking which contributed largely toward the success many of them eventually attained. Further, the

publication of these "Studies" did much to attract attention and arouse interest in a phase of physiological work which neither the chemist nor the physiologist was any too familiar with.

In 1879 *The American Chemical Journal* was established by Dr. Ira Remsen under the auspices of The Johns Hopkins University and with the second volume of this Journal (1880) there was commenced a series of *Reports on Progress in Physiological Chemistry* which were continued in successive volumes for several years. In these *Reports* I endeavored to bring before the chemists of the country many of the new ideas being promulgated, especially by European workers, in the field of physiological chemistry and the facts upon which they were based, under such topics as digestion, metabolism, etc., hoping thereby to arouse interest in the broader aspects of physiological chemistry and to show that here was a field of work which merited wider recognition in the United States.

After 1890 there was a rapid development of physiological chemistry in America, due to a variety of causes. Growth of knowledge, especially along biological lines, was gradually arousing interest in all the experimental sciences and giving them a position not hitherto accorded. Further, the growth of interest in the experimental method, the conviction that students could acquire a first-hand knowledge of scientific principles only by personal experience in the laboratory, was tending to bring about radical changes in the educational system of the country. It was becoming increasingly evident that medical education in America was greatly in need of improvement and

that the biological sciences were a necessary foundation upon which to build the medical superstructure. The time had gone by when the professor of anatomy or physiology in the American medical school could be a general practitioner and at the same time lecture on physiology, for example. The teachers of such subjects as anatomy, physiology, pharmacology, etc., must be men trained in the modern methods of research and so interested in the progress of their particular science that they would of necessity devote all their time and energy to its development. As a result of such convictions, biological courses of instruction were being established in American universities in increasing numbers and medical schools were not only recognizing the importance of such training for the prospective medical student, but there was even a tendency to insist upon such training as a condition of admission.

In physiology especially, laboratories were being created both for instruction and for research, and professors were being appointed on the strength of their scientific training and their ability as productive scholars. The whole atmosphere was changing and an increasing number of men, trained in the experimental method abroad, were being drawn into the work of the American universities and medical schools. One indication of the new point of view was the fact that here and there professors of physiology were being appointed, even in medical schools, who were not doctors of medicine but simply doctors of philosophy. Physiology was gaining ground as an experimental science, bound neither to medicine nor to any other form of application. The conception of

physiology as a true biological science was materially helped, in this country at least, when The Johns Hopkins University was founded in 1876 with a biological laboratory, in which experimental physiology had a conspicuous place under the leadership of H. Newell Martin as professor of biology, and this at a time long before a medical school became a part of the University.

Medical chemistry in the American medical school was giving place to physiological chemistry with its broader conception of the importance of the chemical functions of the animal body, and well-equipped laboratories were being established in many medical schools, manned by professors and associates well trained for experimental work in physiological chemistry. Finally, and most important of all, the fruitful results of workers in physiological chemistry throughout Europe were rapidly winning converts to the view that in this branch of biological science was a field of endeavor rich in possibilities for increase of knowledge and equally rich in the possibilities of aid to medicine, hence the field must be cultivated.

Recognizing the importance of some concerted movement for the advance of experimental physiology in this country, some stimulus for the encouragement of original research, a few interested men gathered together in New York on December 30th, 1887, to discuss the possibility of forming a physiological society "to promote the advance of physiology." Among this small group were Dr. S. Weir Mitchell of Philadelphia; Dr. Henry P. Bowditch, professor of physiology at the Harvard Medical School; Dr. H. Newell Martin, professor of biology at The Johns Hopkins University; Dr. John G. Curtis, professor of

physiology at the College of Physicians and Surgeons, Columbia University; Dr. Horatio C. Wood, professor of therapeutics at the Medical School of the University of Pennsylvania, and Dr. Russell H. Chittenden, professor of physiological chemistry in the Sheffield Scientific School of Yale University. As a result of this conference, *The American Physiological Society* was organized, with S. Weir Mitchell, president, and H. Newell Martin, secretary, the first regular meeting being held in the following year with a small membership.

Today, 1928, the Society has a membership of 403, seventy-four universities and colleges being represented. In addition many research institutions of various types, all doing some form of physiological research, are to be found in the membership. Of the 403 members, 136 are professors, associate professors or assistant professors of physiology in some American university or medical school, while forty-six hold similar rank in physiological chemistry or biochemistry. There is thus indicated something of the phenomenal growth in America of the interest and activity in experimental physiology which has occurred during the past forty years.

In 1896, *The Journal of Experimental Medicine* was established at Baltimore, with William H. Welch as editor. This was the pioneer Journal in the United States "devoted to the publication of papers of a more or less technical or monographic character" in physiology, pathology, pharmacology and medicine. There were four groups of associate editors: for physiology, H. P. Bowditch of Boston, R. H. Chittenden of New Haven and W. H. Howell of Baltimore. In the *Introduction* to the first vol-

ume, it was stated "that contributions to physiology and physiological chemistry shall be a prominent feature of the Journal."

In the first volume the following papers of a chemico-physiological character appeared: *The Mucin of White Connective Tissue,* by R. H. Chittenden and William J. Gies, from the Sheffield Laboratory of Physiological Chemistry; *On the Pigment of the Negro's Skin and Hair,* by John J. Abel and Walter S. Davis, from the Laboratory of Physiological Chemistry of The Johns Hopkins University; *The Immunizing Power of Nucleohiston and of Histon,* by F. G. Novy, from the Hygienic Laboratory of the University of Michigan; *The Rotary Properties of Some Vegetable Proteins,* by Arthur C. Alexander, from the Sheffield Laboratory of Physiological Chemistry; *A Chemical Study of the Secretion of the Anal Glands of Mephitis Mephitica (Common Skunk), with Remarks on the Physiological Properties of This Secretion,* by Thomas B. Aldrich, from the Laboratory of Physiological Chemistry of The Johns Hopkins University.

Another event, suggestive of growth and accomplishment, is to be found in the establishment in 1898 of the *American Journal of Physiology,* edited for the American Physiological Society by a group of seven members of the Society, the first group being composed of Professors Henry P. Bowditch of Harvard University, Russell H. Chittenden of Yale University, William H. Howell of Johns Hopkins University, Frederic S. Lee of Columbia University, Jacques Loeb of the University of Chicago, Warren P. Lombard of the University of Michigan, and William T. Porter of Harvard University.

Not only as a matter of historical interest, but even more of justice, it should be made clear that while the journal was in a sense under the jurisdiction of the Society and controlled by it, its existence really depended upon one man, William Townsend Porter, who gave time, service and money to make the journal possible. Without his generous efforts the journal could not have been established at that date and without his unremitting care and attention it probably never would have reached that perfection of detail in *format* and typography that has always characterized the *American Journal of Physiology.*

Of the thirty-two papers that appeared in the first volume in 1898, seven were from the Sheffield Laboratory of Physiological Chemistry, *i.e.*, were papers on physiological chemistry, and in addition there were two papers of a chemico-physiological character from the Physiological Laboratory of the Yale Medical School. Of the twenty-three papers in the second volume of the journal, eight were in the field of physiological chemistry.

This is sufficient to indicate, I think, that in these movements for the encouragement of experimental physiology in America there was every disposition to consider physiological chemistry as a part of physiology. It was not to be set apart as a thing by itself, neither was it to be looked on as distinctively chemical. The main purpose of physiological chemistry is to study and explain, so far as possible, the chemical functions of the living organism and such being the case, it belongs with, and is a part of, physiology. That such was the view held by a majority of the members of the American Physiological Society is indicated by the fact that for nine years

in succession a physiological chemist served as the president of the Society. There is no disposition to over-emphasize this view, but I believe it important to register the state of mind that has prevailed among American physiologists from the very beginning of activity in this field of research. I believe it equally true that much of the success which has attended the efforts of American workers in physiological chemistry has been due to the close relationship which has always existed between the representatives of physical physiology and chemical physiology. A broader viewpoint, a fuller appreciation of the physiological significance of chemical findings, a clearer understanding of relative values in physiological research, have resulted from the recognized kinship of the two branches of this experimental science.

As the years passed, with increase in the number of workers in physiological chemistry, there came a time, in 1906, when it seemed necessary to form a new society. Specialization of effort was leading to the formation of a relatively large group of men whose interests were wholly in the field of chemical physiology and who could not be given time at the annual meetings of the American Physiological Society for adequate presentation and discussion of the many papers of chemico-physiological significance. As a result, there was created the *American Society of Biological Chemists*, with the avowed purpose "to further the extension of biochemical knowledge and to facilitate personal intercourse between American investigators in biological chemistry." This relieved the pressure in the parent society and gave room for the further

expansion of each under perhaps more favorable conditions.

The close relationship of the two societies to each other and to the *American Society for Pharmacology and Experimental Therapeutics* as well as to the *American Society for Experimental Pathology* was recognized by the establishment in 1913 of the *Federation of American Societies for Experimental Biology*, the total membership of which in 1928 was 1,020; a striking index of the scope and extent of the experimental work in these four allied branches of biological study in America. The point to be emphasized, however, is the element of growth and development that these statements point to, and also the element of interdependence and correlation they so clearly imply. In this same year, 1913, the American Chemical Society authorized the creation of a "Division" of Biological Chemistry.

In 1928, the membership of the *American Society of Biological Chemists* was 330, sixty-six universities and colleges being represented. An analysis of this membership affords a striking illustration of the broad interests covered by physiological or biological chemistry and its many forms of service. Eighty-two members of the society hold positions as professors or assistant professors of physiological chemistry, biological chemistry, or biochemistry in some American university, college or medical school. Somewhat interesting and suggestive, too, is the distribution under the three titles, *viz.*, physiological chemistry 37, biochemistry 30, biological chemistry 15. Again, many research workers in physiological chemistry, members of the Society of Biological Chemists, carry

the title of professor of chemistry, physiology or pharmacology, representing perhaps their line of approach, or their original university appointment. Thus, in the 1928 list of members, there are twenty-two enrolled under chemistry, eighteen under physiology and nineteen under pharmacology.

The point I wish to emphasize especially, however, is the variety in the lines of experimental work carried on by members of the society, not connected with universities or medical schools such as research work in the *Nutrition Laboratory of the Carnegie Institution of Washington,* the *Rockefeller Institute for Medical Research,* the *Otho S. A. Sprague Memorial Institute,* the *Russell Sage Institute of Pathology and the Food Research Institute* at Stanford University, with which eighteen or more members of the society are connected; the various lines of work in the *United States Department of Agriculture,* as protein and animal nutrition, dairying, soil fertility, etc., with twelve members so occupied; the work of the *Agricultural Experiment Stations* in which biochemistry is an important factor, where at least six members of the society are concerned; workers in agricultural chemistry where biochemical research is called for, eleven in number; specialized biochemical research in nutrition, metabolism, etc., with eleven members involved; research work of various types, but of a biochemical character, in *Medical Clinics and Hospitals,* where nineteen members are employed; biochemical research in *Departments of Health,* in *Life Insurance Companies,* in *Pathological Institutes,* where nine members of the society are occupied; research work of a biochemical character in pediatrics,

bacteriology, hygiene, plant physiology, in which fourteen members of the society are engaged; research work in food chemistry, in home economics, in the manufacture of food products, pharmaceutical preparations; biochemical work in various organizations such as the Grain Research Laboratory, National Canners Association, etc.

These statements testify to the diversity of interests that depend in some measure at least upon the help that physiological chemistry can render, which in turn suggests one of the reasons why physiological chemistry has grown so remarkably in America during recent years. To be sure, the physiologist and the physiological chemist interested solely in the development of his science, is not so keenly alive to what is being done around the edges of his domain, although it sometimes happens that even in industrial operations suggestive reactions are brought to light with fruitful results for science. But any form of knowledge that holds out a helping hand, even in a limited way, arouses interest and gains support sometimes of a very substantial character. Physiological chemistry owes much to the friendly help given by those who have benefited from the advance of knowledge and by those who have seen and appreciated the many accomplishments this branch of science has been responsible for.

One cannot think of the American Society of Biological Chemists without taking note of the *Journal of Biological Chemistry,* for which it is responsible. This journal was founded in 1905 by the late Professor Christian A. Herter of New York, a year before the society was established. In 1911, the *Christian A. Herter Memorial Fund* was created and by a trust agreement executed by the *Journal*

*of Biological Chemistry* and the donors of the fund, the income therefrom is to be expended, under the direction of the editorial board for the maintenance and partial support of the journal, the editorial board being composed of four members of the society. A glance through the eighty-four volumes of the journal which have appeared since its establishment affords perhaps the most convincing evidence of the great activity which has prevailed among American workers in the field of physiological chemistry during the past twenty years. It will likewise reveal something of the great breadth of the field of investigation covered by the physiological chemistry of the present day.

The question may be asked, where do all these research workers in physiological chemistry come from? The answer is to be found in the catalogs of American universities and medical schools, in the lists of professors of this branch of science contained therein, in the description of laboratory facilities for advanced and research work, and in the legends under the name of the author in the various contributions found in American journals devoted to the biological sciences. At Yale University the first degree of doctor of philosophy in physiological chemistry was granted in 1880. In the forty-seven years between that date and 1927, this degree has been conferred upon ninety-three candidates, whose advanced work has been mainly in the Sheffield Laboratory of Physiological Chemistry. In addition, the Master's degree has been given to various candidates whose work had not been sufficiently advanced or extensive to warrant the higher degree.

It may be interesting to note that of these ninety-three

doctors of philosophy, twenty hold, or have held, professorships of physiological chemistry, biological chemistry or biochemistry in American universities or medical schools, while fourteen hold corresponding positions under the title of nutrition or home economics. In addition, nine occupy chairs which carry the title of professor of chemistry, although their work and their interests are mainly in physiological chemistry; six are professors of physiology; two, professors of clinical medicine; three, professors of pharmacology; two, professors of bacteriology; and one, professor of biology. Further, a number hold responsible positions as biochemists in research institutions of varying character.

What has been written of Yale may be duplicated, in some measure at least, by other universities which have developed and encouraged advanced work in physiological chemistry, and the number of such is impressive. The need to look to European laboratories for training and experience in physiological chemistry, so conspicuous in 1878, no longer exists, for today the American universities provide all necessary facilities in modern and thoroughly equipped laboratories, and furnish guidance and inspiration through competent leaders who are themselves highly productive scholars.

What has been written in this chapter indicates in a general way the character and extent of the development in physiological chemistry which has taken place in America during the past fifty years. Teaching and research have been developed side by side in our universities, while the many research institutions which have come into existence have given American scientists added

opportunities for carrying on the most abstract and profound research in experimental physiology, physiological chemistry and scientific medicine. Naturally, there has come growth and development. Laboratories, equipment, societies and journals are important and essential, but increase of knowledge comes mainly from the minds of men and their activities. These latter, it will be important to trace out and analyze if we are to have a clear picture of the gradual development of physiological chemistry in America to its present-day standing. It will be necessary, therefore, to go back to the beginning of many things; to follow from their sources the currents of activity, which once started have flowed steadily on, bringing results of varying importance to physiological knowledge.

# CHAPTER III

Early lines of experimental work—Nutritive value of food materials —Work of Wilbur O. Atwater and collaborators—Dietary habits of the American people—The Atwater-Rosa calorimeter—Work of Henry P. Armsby at Pennsylvania State College—Studies of Francis G. Benedict of the Nutrition Laboratory of the Carnegie Institution of Washington—Calorimetric work of Graham Lusk of Cornell University Medical College—Studies of Eugene F. DuBois at the Russell Sage Institute—Metabolic studies on diabetics by Elliott P. Joslin—Chittenden's experiments on protein requirements—Studies by Benedict and others on efficiency under restricted diet—The work of Otto Folin of the Harvard Medical School.

Among the early lines of experimental work that aided in the gradual evolution of physiological chemistry in America, studies in the field of nutrition stand out with great distinctness. The processes of nutrition, however, are many; indeed, so broad is the term that included under it are to be found many related topics, each one of which contributes something to that total of knowledge that enlightens our understanding of the subject. Food requirements, the chemical composition of foods, normal diet, the nutritive value of different foods, protein requirements, specific dynamic action, nitrogen equilibrium, calorimetry, general metabolism, intermediary metabolism, basal metabolism, metabolism in disease, are but a few of the different lines of inquiry that have engaged the attention of workers in the field of nutrition, development of which

has brought our knowledge to the position it now occupies.

Up to about 1880 anyone desiring information regarding the chemical composition and nutritive value of food materials had to depend mainly upon the analyses of European products, the standard authority being König's *Chemie der Menschlichen Nahrungs-und Genussmittel.* In this country, while there were isolated studies of American food products, as in the investigations carried out by Professor Atwater, 1878-1881, under the auspices of the United States Fish Commission [1] and the Smithsonian Institution, it was the work largely of the agricultural experiment stations of various states, notably Connecticut, and particularly the Office of Experiment Stations, United States Department of Agriculture (established 1888), of which Professor Atwater was the first director, that systematized and broadened knowledge of the chemical composition of American foodstuffs.

In the Bulletin (No. 28) of the United States Department of Agriculture, by W. O. Atwater and Chas. D. Woods, published in 1896, there are recorded approximately 2,600 analyses of American products representing all types of animal and vegetable foodstuffs, showing the content of water, protein, carbohydrate, fat and ash, together with the fuel value per pound, over one thousand of the analyses having been made by Atwater and his associates. Later, in 1899, Atwater and Bryant issued supplementary tables in which were recorded the results of the analyses of some 4,000 specimens of American food-

[1] W. O. Atwater, "Contributions to the Knowledge of the Chemical Composition and Nutritive Values of American Food Fishes and Invertebrates."

stuffs. Thus was rendered available a mass of material of great value to all interested in nutritive values and in dietary studies.

Wilbur Olin Atwater was trained in agricultural chemistry, taking the Ph.D. degree in the Sheffield Scientific School at Yale in 1869 under Professor Samuel W. Johnson, after which he studied for a time at Leipzig and Berlin, becoming professor of chemistry at Wesleyan University in 1873. He was a man of wide vision and he clearly saw the possibilities of chemical aid in the study of human and animal nutrition. He was an outstanding representative of a fairly large group, who, trained in chemistry, sooner or later, took up the study of problems more or less closely related to physiology. Atwater soon became interested in the study of dietary customs and habits, stating in one of his papers published in 1898, "It is by means of dietary studies that the most reliable data concerning the food consumption of people of different nationality, sex, and occupation, and under different financial and hygienic conditions, can be obtained." As special agent in charge of nutrition investigations carried on by the Office of Experiment Stations he made with the coöperation of various assistants a number of dietary studies, the analytical work being done mainly at Wesleyan University, Middletown, Connecticut.

As an example of this form of investigation, reference may be made to Bulletin No. 55, United States Department of Agriculture, *Dietary Studies in Chicago in 1895 and 1896,* "conducted with the coöperation of Jane Addams and Caroline L. Hunt of Hull House," reported by W. O. Atwater and A. P. Bryant. The main object of

this investigation was "to obtain information regarding the conditions of living and the peculiar economy of the food of the poor of different nationalities residing in the worst congested districts of Chicago." Consequently, such a study served a two-fold purpose, *viz.*, throwing light upon social and economic conditions and contributing something to knowledge of physiological value.

About this period, 1894-1897, a large number of similar studies bearing on the food and nutrition of man were carried on by various investigators more or less under the jurisdiction of the Office of Experiment Stations and published in Bulletins of the United States Department of Agriculture, such as C. E. Wait, *Dietary Studies at the University of Tennessee in 1895*, Bulletin No. 29; H. B. Gibson, S. Calvert, and D. W. May, *Dietary Studies at the University of Missouri in 1895, and Data Relating to Bread and Meat Consumption in Missouri*, Bulletin No. 31; E. B. Voorhees, *Food and Nutrition Investigations in New Jersey in 1895 and 1896*, Bulletin No. 35; A. Goss, *Dietary Studies in New Mexico in 1895*, Bulletin No. 40; W. O. Atwater, and C. D. Woods, *Dietary Studies in New York City in 1895 and 1896*, Bulletin No. 46; Isabel Bevier, *Nutrition Investigations in Pittsburgh, Pa., 1894-1896*, Bulletin No. 52.

By studies of this character much knowledge was gained regarding the dietary habits of the American people under different conditions of life, and following the lead of European physiologists and chemists who had made many similar studies in European countries, there was a disposition to draw more or less definite conclusions regarding the food requirements of the human body

under different conditions of age and activity. Thus, there arose the so-called Atwater "standard diet" for a man of 70 kilograms body weight; doing light work, 100 grams of protein per day with fats and carbohydrates sufficient to make a total of 2,700 calories; doing medium work, 125 grams of protein with a total of 3,400 calories; doing hard work, 150 grams of protein with a total of 4,150 calories.

In all, some 350 such dietary studies of several thousand people in various parts of the United States were made, in all of which Professor Atwater was more or less directly concerned. While it may perhaps be questioned whether the scientific value of this extensive inquiry was sufficiently great to justify the large expenditures involved, it is certainly true that there was aroused a widespread interest in dietary matters, leading the people to think in terms of nutritive values and to give more heed to their own nutrition. Possibly of greater value was the influence this aroused interest had in determining the action of the United States Government in authorizing and supporting a more general inquiry, of greater physiological importance, regarding the food and nutrition of man.

While studies such as those just referred to gave a fairly accurate picture of the nitrogen or protein consumption per day, there remained much to learn regarding the metabolic processes of the body under different physiological conditions. Atwater was ambitious to emulate Rubner's work with the respiration-calorimeter, to determine the heat value of the different foodstuffs as they serve their purpose in the body, to measure the

heat production in man, to prove and confirm the general laws of metabolism in the human organism. This was not easy of accomplishment, but after several years of effort, 1892-1897, the desired respiration-calorimeter was successfully completed, at Wesleyan University, largely through the skill of Professor E. B. Rosa, at that date professor of physics at the university, the necessary expense being met mainly by the United States Government.

The Atwater-Rosa calorimeter, the first apparatus of the kind to be used in America, was not only large enough to measure with a high degree of perfection the amount of heat given off by a man at rest or at work, but it was also possible to measure the oxygen consumed, being in many respects "the most important form of respiration-calorimeter yet devised." The accuracy of the apparatus was remarkable as was shown by burning a given weight of ethyl alcohol in the chamber, the carbon dioxide recovered being 99.8 per cent of the theoretical value, and the heat production 99.9 per cent. With this apparatus was inaugurated a series of investigations of the greatest physiological value in the study of human nutrition, a credit to American enterprise and scientific acumen. The earlier results were published in the Bulletins of the *Office of Experiment Stations,* 1897-1902, Nos. 44, 45, 69, 109, 136, under the title *Experiments on the Metabolism of Matter and Energy in the Human Body,* by W. O. Atwater and F. G. Benedict, with the coöperation of a number of associates.

Their studies with this respiration-calorimeter confirmed many of the findings of European physiologists and

at the same time gave added data of physiological value for the solving of problems in nutrition. Thus they showed that the heat produced by a man in a given period of time is the same in quantity as that which can be derived from the energy liberated in the oxidation of food materials during the same period; that the energy which a man expends at hard work, is the exact equivalent of the energy liberated by the body metabolism. Their results confirmed the views held by physiologists generally that the energy of muscular work comes mainly not from the oxidation of protein matter, but from the oxidation of carbohydrates and fats, thus affording added proof of the incorrectness of the old theory that protein was the sole source of the energy of muscle work. Especially noteworthy is the fact that these investigations demonstrated for the first time on *man* the application of the principle of the conservation of energy to the human organism.

Especially important was a study of the *Nutritive Value of Alcohol,* one of the investigations made under the direction of a sub-committee of the "Committee of Fifty" to study the *Physiological Aspects of the Liquor Problem,* the sub-committee being W. O. Atwater, John S. Billings, H. P. Bowditch, R. H. Chittenden and W. H. Welch. This extensive investigation conducted with the respiration-calorimeter at Wesleyan University by Professors Atwater and Benedict assisted by a corps of assistants, afforded convincing proof that "alcohol is similar to the fuel ingredients of ordinary food, the carbohydrates and fats, in that it is oxidized in the body, yields energy for warmth and probably for work, and protects

body material from consumption." As regards efficienc in protecting body protein, the carbohydrates and fat outrank alcohol, while the relative fuel value of alcoho is expressed by the statement that 4 grams of alcohol are isodynamic with 7 grams of sugar or starch and with 3 grams of fat.

It is interesting to note that another impetus to the study of nutrition came from an agricultural chemist, Henry P. Armsby, an ardent worker in the field of animal nutrition. A graduate student in the Sheffield Scientific School at Yale, under Professor Samuel W. Johnson, he gained the Ph.D. degree in chemistry in 1879, becoming eventually the director of the Pennsylvania State College Institute of Animal Nutrition. For Armsby's work the United States Department of Agriculture furnished funds for the construction at State College of a special calorimeter of the Atwater-Rosa type adapted for use with farm animals, even as large as cattle; said to be at present the only apparatus of its type in existence.

Commencing in 1901 Armsby, by the use of this apparatus, conducted many series of experiments which led to accurate determinations of the energy requirements of livestock, which in turn led to the more scientific feeding of farm animals. Especially valuable was his use of net energy as a means of determining the true nutritive value of food materials for maintenance and production. While Armsby's work was almost wholly in the field of animal nutrition, yet much that he accomplished was, indirectly at least, of service to mankind. Thus, he stressed the great importance of a proper adjustment of human and animal foodstuffs with a view to saving energy lost through feed-

ing to farm animals products quite suitable for direct consumption by man. Moreover, his investigations demonstrated for the first time the application of the principle of the conservation of energy to large farm animals.

Whenever there is any discussion regarding problems connected with the metabolism of matter and energy in the human body, the name of Francis G. Benedict will appear. Associated with Atwater at Wesleyan University as professor of chemistry, also as physiological chemist of nutrition investigations of the United States Department of Agriculture, he was responsible for much of the success that attended the experiments with the respiration-calorimeter at Middletown. A graduate of Harvard University, A.B., 1893, and a student of chemistry at Heidelberg, 1895, he became in 1907 the director of the newly established *Nutrition Laboratory of the Carnegie Institution of Washington,* and with all the facilities provided by the commodious Boston laboratory of that institution, together with the abundant resources at his command, Benedict has been able to contribute much to our knowledge of human nutrition and has helped thereby to enhance greatly the position of America in the field of physiological chemistry.

A long series of publications have come from the Carnegie Institution during the past twenty years containing the results of the many studies that have been carried on by Benedict and his associates, among which may be mentioned *The Influence of Inanition on Metabolism,* 1907; *The Metabolism and Energy Transformations of Healthy Man During Rest,* with Thorne M. Carpenter, 1910; *Metabolism in Diabetes,* with Elliott P. Joslin, 1910; *A*

*Study of Metabolism in Severe Diabetes,* with Elliott P. Joslin, 1912; *Muscular Work; A Metabolic Study with Special Reference to the Efficiency of the Human Body as a Machine,* with Edward P. Cathcart, 1913; *Factors Affecting Basal Metabolism,* 1915; *Energy Transformations During Horizontal Walking,* with Hans Murschhauser, 1915; *The Physiology of the New Born Infant, Character and Amount of the Katabolism,* with Fritz B. Talbot, 1915; *A Study of Prolonged Fasting,* 1915; *Food Ingestion and Energy Transformations with Special Reference to the Stimulating Effect of Nutrients,* with Thorne M. Carpenter, 1918; *Human Vitality and Efficiency under Prolonged Restricted Diet,* with Walter R. Miles, Paul Roth and H. Monmouth Smith, 1919; *A Biometric Study of Basal Metabolism in Man,* with James Arthur Harris, 1919; *Metabolism and Growth from Birth to Puberty,* with Fritz B. Talbot, 1921.

From these titles something of the wide scope of Benedict's studies on human nutrition will be gleaned. Obviously, it is quite impossible to give here any adequate review of this great volume of work which has been such a credit to American physiology. Attention may be called, however, to several findings of special physiological significance. Thus, in his study of prolonged fasting, one subject, a man with an initial body weight of 59.6 kilograms, who went without food for thirty-one days, lost a total of 12.2 kilograms in weight, and 277 grams of body nitrogen, the equivalent of 1,731 grams of protein. On the thirty-first day of the fast the output of nitrogen was 6.94 grams, equal to 43.3 grams of protein, while the calories amounted to 1,072. On the preceding day, in the

bed calorimeter, the average heat production was the lowest recorded, *viz.*, 1,025 calories for the twenty-four-hour period. It is to be noted that there were no permanent deleterious effects as a result of the fast that could be discovered, either in muscular strength or in mental activity.

Again, it is well known that in fasting, the losses of nitrogen during the early days of the fast depend in considerable degree upon the amount of glycogen stored up in the body, and on the quantity of protein food consumed just prior to the fasting period. In other words, the metabolism of glycogen and of the sugar which comes from it tends to protect the metabolism of protein. This influence was well shown in one of Benedict's experiments, where on the first day of the fast 181.6 grams of glycogen were metabolized and 5.84 grams of nitrogen eliminated, while on the second day the glycogen metabolized amounted to only 29.7 grams but the nitrogen excreted increased to 11.04 grams.

Especially interesting and important are Benedict's studies on basal metabolism; *i.e.*, the energy requirements of the quiescent body. It would seem at first glance that the establishment of a base-line would be comparatively simple but such is not the case, as there are so many factors involved, age, weight, sex, the character of the resting, whether in bed or sitting in a chair, body surface, period of digestion, etc. Benedict's many results, a great mass of material representing metabolic studies carried out under a wide range of conditions, have, however, provided standards for measuring the basal metabolism. The extensive studies (with Talbot) of the metabolism of

children from infancy to maturity have likewise furnished standards of value. Reference may also be made to several papers which appeared in the *Journal of Biological Chemistry* in 1915: *A Comparison of the Basal Metabolism of Normal Men and Women*, by Benedict and Emmes; *Metabolism of Vegetarians*, by Benedict and P. Roth; *Metabolism of Athletes as Compared with Normal Individuals of Similar Height and Weight*, by Benedict.

While it would appear that the level of basal metabolism is dependent upon the bulk or mass of the active protoplasm yet, as expressed by Benedict (*J. Biol. Chem.* XX: 1915), "The basal metabolism of an individual is a function first, of the total mass of active protoplasmic tissue, and second, of the stimulus to cellular activity at the time the measurement of the metabolism is made. Apparently, at present no law can be laid down that will cover both of these important variables in the basal metabolism of an individual." Still, as the many results show, there is generally a certain definite relationship between the amount of heat produced and the surface area of the body, in harmony with Rubner's original law "that the metabolism is proportional to the superficial area of an animal." Thus, with thin women nearly fifty per cent more heat was found than with obese women, still per square meter of surface there was not much if any difference; a result that accords with Rubner's well-known findings with a fat boy and his older but lean brother.

While it is probably true that the height of basal metabolism is determined by the mass of active protoplasmic tissue, it is obvious that this mass cannot be measured.

To find the basal metabolism, without actual calorimetric determination, Benedict and Harris devised certain multiple prediction tables which are more or less generally used, but they do not appear to offer any great improvement over the formulas based on surface area, such as recommended by DuBois.

Finally, we may refer to the interesting and suggestive studies by Benedict and Carpenter on *Food Ingestion and Energy Transformations,* noting particularly the experiments with mixed diets in which it was found that with excessive amounts of food there was a stimulation of metabolism, amounting "to 40 per cent above the basal value for a number of hours and to 20 per cent for at least 8 hours; indeed there was every reason to believe that the stimulus to the metabolism would have been found to continue considerably longer than the experimental period of 8 hours if the observations had been prolonged. This fact has a special practical significance in its relation to the daily life of human individuals. While it is possible for a human being to live with greatly reduced activity when sound asleep, without food in the stomach, and without extraneous muscular activity, his efficiency as a member of human society in such a state would be negligible. It is therefore only as the cellular activity increases that we find him becoming more and more of service to humanity, and not until he is erect and ready to perform active external muscular work is he in a condition to live on a basal plane that is of practical value."

Another chapter in the study of calorimetry in America is bound up with Graham Lusk, professor of physi-

ology in the Medical College of Cornell University and scientific director of the Russell Sage Institute of Pathology. Lusk was trained as a chemist at Columbia University, Ph.B., 1887, and later, 1891, took the Ph.D. degree under Baeyer of Munich, after which he worked with Carl Voit, where he acquired his knowledge of physiology and his interest in nutrition. For seven years he was in the Yale Medical School, first as instructor, later as professor of physiology. From 1898 to 1909 he was professor of physiology in the University and Bellevue Hospital Medical College. When he assumed his duties at the Cornell Medical College in 1909 he was provided with funds for the construction of a respiration apparatus sufficiently large to measure the respiratory metabolism and heat production of dogs and babies.

A description of this calorimeter by H. B. Williams, now Dalton professor of physiology at Columbia University, was published in 1912 as the first of a series of studies on calorimetry from Lusk's laboratory. With this apparatus a long series of experiments of varied character under the general title of *Animal Calorimetry* have been carried on by Lusk and his co-workers during the past twenty years, with results of great physiological importance. Of equal importance was the installation by the Russell Sage Institute of Pathology of a large respiration-calorimeter in Bellevue Hospital, where Professor Eugene F. DuBois, the medical director of the Russell Sage Institute, with the aid of a full-time staff, conducted many experiments on the metabolism of healthy people and on hospital patients. It is stated that this was "the first time a respiration-calorimeter was built in a hospital."

Many studies were made by Lusk and his associates using the small respiration chamber, and by DuBois with the larger apparatus at Bellevue Hospital, with a view to establishing normal standards of basal metabolism; all their results, obtained with adults, children, babies and dogs testifying to the striking uniformity of the law of surface area. The late John Howland, working with infants five to seven months old (1910-1911) found that the heat production of the sleeping infant reached a higher level per square meter of surface than that of adults, thus implying a higher metabolism in the youthful protoplasm of the infant. In crying, the calories per square meter of surface were greatly increased, thus showing accelerated metabolism as Murlin and others have observed. The work of Benedict and Talbot, as well as the studies of Katherine Blunt and her associates, and of DuBois, all agree in indicating that the general trend of metabolic activity is downward from the early years of childhood to the adult stage, *i.e.*, the basal metabolism grows progressively lower. During the first few months of life, however, as Murlin and others have shown, metabolism per square meter of surface is increased. In this connection Murlin has suggested that coördination between heat production and heat loss is not well developed at birth or shortly thereafter.

Lusk in his studies of animal calorimetry,[2] using dogs, has worked on such subjects as *Metabolism of the Dog following the Ingestion of Meat in Large Quantity,* with H. B. Williams and J. A. Riche, 1912; *Metabolism after the Ingestion of Dextrose and Fat,* 1912; *The Influence*

[2] Published in *J. Biol. Chem.*

*of the Ingestion of Amino-acids upon Metabolism*, 1912; *The Influence of Mixtures of Foodstuffs upon Metabolism*, 1912; *An Investigation into the Causes of the Specific Dynamic Action of the Foodstuffs*, 1915; *The Influence of the Ingestion of Fat*, with J. R. Murlin, 1915; *The Interrelation between Diet and Body Condition and the Energy Production during Mechanical Work*, with R. J. Anderson, 1917; *Further Experiments Relative to the Cause of the Specific Dynamic Action of Protein*, with H. V. Atkinson, 1918; *Influence of Lactic Acid upon Metabolism*, with H. V. Atkinson, 1919; *On the Problem of the Production of Fat from Protein in the Dog*, with H. V. Atkinson, 1920; *The Behavior of Various Intermediary Metabolites upon the Heat Production*, 1921; *The Production of Fat from Protein*, with H. V. Atkinson and David Rapport, 1922; *The Influence of Glycyl-Glycine upon the Respiratory Metabolism of the Dog*, with Norman H. Plummer and Harry J. Deuel, Jr., 1926; this being the thirty-fourth paper from Lusk's laboratory on animal calorimetry.

Lusk's experiments with dextrose showed that during the first four or five hours after the ingestion of this sugar (50-100 grams) the heat production rises 20 per cent above the basal metabolism, due, as he believed, to the presence of a greater amount of free diffusible carbohydrate, in harmony with the "older" view of Voit that the presence of abundant food increases the power of the cells to metabolize. In the experiments (with H. B. Williams and J. A. Riche) where large quantities of meat were fed it was found that "the increased metabolism was proportional to the nitrogen elimination except in the

*second and third hours. In the second hour the metabolism rose almost to its maximum although* the urinary nitrogen reached only a third of its maximum. Since the non-protein respiratory quotient for this period was often above 0.90, it appears that carbohydrate and not additional protein was oxidized during this hour. On this is based the argument that the incoming amino-acids, in proportion to their mass action stimulate the protoplasm to higher oxidation." That intestinal work has little to do with the increased heat production would appear probable from the fact that a high metabolism was maintained hours after the food had been digested and three-quarters of the nitrogen of the protein had been eliminated through the urine.

Especially important were the experiments with amino-acids, in which it was shown that glycocoll and in some degree alanine and leucine, in contrast to glutamic acid, act as stimuli to increase the oxidative processes in the organism, glycocoll, in particular, augmenting greatly the heat production. Since glutamic acid and aspartic acid apparently exert no specific dynamic action Lusk concluded that the increased metabolism was due to the direct stimulus of glycocoll and not to any process concerned with intermediary metabolism such as the process of deaminization, a view which is strengthened by the more recent findings of Rapport and Beard (1927) that phenyl-alanine has a greater specific dynamic action than any other amino-acid.

From these and the results of other experiments, Lusk was strengthened in his belief that the increase in metabolism after the ingestion of meat is due to the mass action

of the amino-acids formed in digestion acting as stimuli upon the cellular protoplasm. In this connection, experiments from Lusk's laboratory by Sophia A. Taistra, 1921, in which acid substances were administered to dogs, showed that "the specific dynamic action of the foodstuffs is not dependent upon the level of the alkaline reserve of the blood plasma as measured by its $CO_2$-combining power." Further, Alfred Chanutin, 1921, working in the same laboratory found that the amino-acids formed after the ingestion of meat do not so reduce the alkaline reserve of the blood that it becomes a contributing cause of the great increase in heat production.

Again, Lusk's work (with R. J. Anderson) with dogs confined in a treadmill fitted within the calorimeter, led him to the conviction that it is the condition of the body and not a large influx of food on the day previous that determines the height of the basal metabolism. Further "when mechanical work is accomplished after high protein ingestion, there is an exact summation of the increment due to the specific dynamic action of meat and that due to energy necessary for the mechanical work involved." Finally, it should be added that Lusk's book, *The Science of Nutrition* (first edition 1906), has been a constant source of help and inspiration to all workers in the field of nutrition.

The studies of Eugene F. DuBois and his associates, among whom may be mentioned Warren Coleman, F. C. Gephart, David P. Barr, W. H. Olmstead, J. C. Aub, Frederick M. Allen, R. L. Cecil, with the Russell Sage respiration-calorimeter at Bellevue Hospital, have covered a wide field, especially on metabolism in disease.

Their results (1916-1918) have likewise furnished a large number of data on the basal metabolism of American children, which with corresponding data on normal adults led to the development by Delafield DuBois and Eugene F. DuBois of a height-weight formula for determining the surface area of the body as a means of estimating or predicting the basal metabolism. The surface area formula of DuBois, previously referred to, is quite generally used, though subject to criticism by some physiologists. "While it may be true that the basal metabolism is not strictly proportional to, nor, perhaps, determined by surface area, the fact remains that it is more nearly proportional to area than to any other one factor so far discovered." Full discussion of this subject and other related matters are set forth in Eugene F. DuBois' book, *Basal Metabolism in Health and Disease*, 1924.

The so-called Sage Institute normal standards devised by Aub and DuBois are based on the hypothesis that metabolism is proportional to the surface area but changes with age and is lower in women.

CALORIES PER SQUARE METER OF BODY SURFACE PER HOUR (HEIGHT-WEIGHT FORMULA)

| *Age, Years* | *Males* | *Females* |
|---|---|---|
| 14 to 16 | 46.0 | 43.0 |
| 16 to 18 | 43.0 | 40.0 |
| 18 to 20 | 41.0 | 38.0 |
| 20 to 30 | 39.5 | 37.0 |
| 30 to 40 | 39.5 | 36.5 |
| 40 to 50 | 38.5 | 36.0 |
| 50 to 60 | 37.5 | 35.0 |
| 60 to 70 | 36.5 | 34.0 |
| 70 to 80 | 35.5 | 33.0 |

In the study of metabolism in disease DuBois and his co-workers have brought many interesting facts to light. Thus, in 1921 it was pointed out by DuBois that in fevers, the increase in metabolism with increased temperatures follows van't Hoff's law. The latter reads "with a rise in temperature of 10 C. the velocity of chemical reactions increases between two and three times. In other words, the coefficient is between 2 and 3." DuBois plotted the relation of basal metabolism to temperature in six different fevers and found a striking parallelism between the body temperature and the height of the total metabolism, although the fevers were the result of various infections. As he stated, the coefficient between 2 and 3 "means an increase of 30 to 60 per cent for the 3 degrees rise from 37° to 40° C. Practically all of the fever experiments are within these limits and the average line shows a temperature coefficient of 2.3."

In diabetes, Elliott P. Joslin beginning his work in the Carnegie Nutrition Laboratory with Benedict brought together his observations on 113 diabetics, in which a total of 661 metabolic experiments were carried out, with 2,219 individual periods, recorded in the publication of the Carnegie Institution and in his book, *The Treatment of Diabetes Mellitus,* 1923. These and many other studies of metabolism in disease by a large number of workers, were, in part at least, the result of the realization in America about 1912 of the great clinical advantages which might accrue by careful studies with the respiration-calorimeter.

Obviously, large forms of this complicated apparatus could not be installed in many places, neither could the necessary skill and experience be easily acquired, nor the

large financial support provided. As a result, many small and simple forms of apparatus, portable, and especially adapted for hospital service have been devised, such as the *Benedict Universal*, the *Benedict Portable*, the *Benedict-Collins* and numerous others. The introduction of a portable respiration apparatus, largely the design of Benedict, has made *indirect* calorimetry an almost universally applicable aid in diagnosis as well as in scientific research. Today there are probably several thousand simple forms of calorimeter distributed throughout America in the wards of hospitals, in research laboratories, in educational institutions, by the skillful use of which many results of physiological and clinical value have been obtained. Some of these results we shall have occasion to refer to later.

Many European investigators, notably Voit of Germany, Hultgren and Landergren of Sweden, Gautier of France, Erisman of Russia and Lichtenfelt studying the diet of Italians, established certain dietary standards for the peoples of these different countries, based on their food consumption under varying conditions of activity. Thus arose the conception of various "normal" or "standard" diets, in a manner similar to the creation of the Atwater "standard diet," already referred to. The physiologist might well question whether customs and habits necessarily afford data upon which to base a judgment of the food requirements of the normal man. Customs and habits might well lead to a food consumption far beyond the physiological necessities of the body. It might even be that a daily food intake much greater than the needs of the body call for would eventually be detrimental rather than beneficial. Since it is a well-established fact

that increase in the amount of protein food is followed by a corresponding increase in the excretion of nitrogen, it follows that the body does not under ordinary conditions store up the excess of protein food but rapidly eliminates the nitrogen-containing portion of the molecule.

Again, since various observers have found that it is possible to maintain, for a brief period, a condition of nitrogen equilibrium on a diet of protein far lower than the 118 grams of protein food per day called for by the generally adopted standards, it seemed wise to study what might be termed the minimum protein requirements of the body, consistent with the maintenance of health and strength. Such a study was commenced by Chittenden in the Sheffield Laboratory of Physiological Chemistry at Yale in 1902, the results being published in a book entitled *Physiological Economy in Nutrition*, 1904, also *The Nutrition of Man*, 1907. The investigation was made possible by substantial grants from the funds of the National Academy of Sciences, from the Carnegie Institution of Washington and from other sources. In addition, the War Department of the United States met in large measure the expense of maintaining at New Haven a detachment of volunteers from the Hospital Corps of the United States Army, detailed by the Surgeon General to serve as subjects of one of the groups. The experiments were carried on with the coöperation of a large staff of chemists and physiologists, among whom Professor Lafayette B. Mendel and Dr. Frank P. Underhill should have special mention.

The subjects of these studies were in three groups: five professors and instructors in the Sheffield Scientific School

representing men of mental rather than of physical activity; eight university students trained in athletics; twenty army men, living under military control with regular drill and gymnasium work. The experiment was continued with most subjects for a period of six months, with careful measurements of intake and output, with nitrogen balances at regular intervals, medical examinations and suitable tests of physical fitness.

The results obtained may be summarized as follows: in the professional group, the man of the smallest body-weight,[3] 57 kilograms, showed for nearly nine consecutive months an average daily metabolism of 5.7 grams of nitrogen or 0.1 gram of nitrogen per kilogram of body-weight, with an average *daily fuel value* of the food of 1,549 calories, as calculated from its chemical composition. As body-weight and nitrogen equilibrium were both maintained, with health and strength apparently normal, it seems proper to assume that the needs of the body for protein food were fully met in this case by a metabolism of 33.75 grams of protein per day, and this without any excessive consumption of non-nitrogenous food. The average result for the members of the professional group was 0.118 gram of metabolized nitrogen per kilogram of body-weight, which for a man of 70 kilograms would mean a metabolism of 51.62 grams of protein per day. The aver-

[3] His original weight was 65 kilograms, but after losing 8 kilograms during the preliminary period, he remained constant at 57 kilograms for several years. Other members of the professional group lost 4-6 kilograms body-weight on the restricted diet, but eventually their weight was maintained at a definite point. With the soldier and athlete groups, losses of body-weight were not so great, in most cases 2-4 kilograms. In several cases there was no loss whatever.

age energy value of the daily food of this group during the balance periods was 2,075 calories.

With the army group the result was essentially the same, the members of this group living for a period of five months without discomfort and without detriment to health and with maintenance of nitrogen equilibrium on amounts of protein food less than one-half that called for by the ordinary dietary standards. With the group of athletes the metabolized nitrogen per kilogram of body-weight amounted to 0.127 gram per day, as the average for the group, which for a man of 70 kilograms would mean a metabolism of 55.5 grams of protein per day, while the estimated energy value of the food averaged 2,580 calories. With health, strength and vigor maintained, in a condition of nitrogen equilibrium, and with no visible impairment of the functions of the body in any of the groups, the conclusion was drawn that man may easily adjust himself to a lowered intake of protein food, with a protein metabolism corresponding to 0.12 gram of nitrogen per kilogram of body-weight.

A somewhat similar investigation, though more extended in scope, but not covering as long periods of time, was carried out by Benedict, Miles, Roth and Smith in 1917-1918, the results being published in a large volume entitled, *Human Vitality and Efficiency under Prolonged Restricted Diet,* 1919, already mentioned. This valuable and exhaustive study of the effects of a restricted diet involved extensive measurements of the gaseous metabolism during rest and at work, psychological measurements, physical activity and endurance, nitrogen metabolized, nitrogen balance, etc. The average nitrogen excretion per

twenty-four hours of one group of twelve men (college students) during a period of two months was 10.5 grams, a value to be taken "as an approximate indication of the level of nitrogen upon which these men were capable of living with their lowered intake." Regarding gaseous metabolism it was found that the net calories required for maintenance at the lower weight level, reached during the period of greatest food restriction, had been reduced from "a net intake of not far from 3,100 or even more calories to a net intake of 1,950 calories." During the entire period of restricted diet, 80 days, the twelve subjects lost a total of 130 to 250 grams of nitrogen without, however, any profound disturbance. The loss in body-weight in Squad A, twelve men, averaged 10.5 per cent, while in Squad B, also twelve men, the average loss was 6.5 per cent.

The authors concluded that the loss of nitrogen was directly associated with a loss of body-material "not simply nitrogenous matter but that which results in an ultimate material lowering of the body-weight." In other words, such loss of nitrogen as occurred did not represent the disintegration of organized body-tissue. A plus nitrogen balance was occasionally found with some of the subjects, but on most days the balance was minus. In this connection it is to be noted that the net energy of the food during the balance periods was very low, thus in one balance period of three weeks, with twelve subjects where the average daily loss of nitrogen per man was 3.10 grams, the net energy of the food averaged only 1,375 calories, the nitrogen content of the food averaging 8.19 grams per day. Of interest is the calculation made by

Benedict of the probable average heat output per 24 hours during the last three days of the experiment with Squad A, the average gross intake being 2,486 calories.

| | Calories |
|---|---|
| Basal heat per 24 hours (computed) | 1,367 |
| Cost of digestion | 149 |
| Heat output due to sitting | 63 |
| Heat output due to walking | 375 |
| *Heat output due to exercise greater than walking* | 228 |
| Total | 2,182 |
| Average net calories per day | 2,245 |

These men, therefore, during the last three days of the diet were subsisting upon one-third less than the amount usually required; but of greater significance is the implication "that there must have been a proportionately great reduction of the energy demands for work other than the basal maintenance." It is impossible here to call attention to all the findings in this important physiological study, but mention may be made of one or two conclusions drawn by the authors. "A comparison of the nitrogen excretions in the urine at these different weight levels is of great significance as indicating the possibility of a material reduction of protein in the diet. . . . We have no reason to believe that a somewhat lower protein level might not have readily been obtained without a correspondingly great increase in calories. The fact that nitrogen equilibrium, or at least an indication of nitrogen equilibrium in the frequent appearance of plus values at the lower weight level, was obtained with the surprisingly low caloric intake of 1,950 calories is, we believe, a

new feature." Finally, the following statement may be added: "A nitrogen excretion with normal men of nine grams of nitrogen, *i.e.*, 0.15 gram per kilogram of body-weight, is a minimum level certainly well above any danger-line," consequently "protein curtailment is an assured and physiologically sound procedure."

On completion of the new buildings of the Harvard Medical School in 1906, commodious laboratories for physiological chemistry were provided and the following year Dr. Otto Folin was appointed professor of biological chemistry, coming to Harvard from the McLean Hospital of Waverly, Massachusetts, where he was engaged as research chemist. Thus were created conditions most favorable for the development of physiological or biological chemistry in this educational center, for the marked ability of Folin as a research worker in this field was already clearly established. His education and training in Chicago University and in the universities of Germany and Sweden had given him a broad experience and equipped him thoroughly for the experimental work he was to carry on. His researches cover a wide range of topics in the field of nutrition, especially certain phases of protein metabolism, and he excelled in devising new and quantitative methods for the study of biochemical questions.

The following papers may be cited as illustrating something of the scope of his studies: *Approximately Complete Analyses of Thirty Normal Urines*, 1905; *Laws Governing the Chemical Composition of Urine*, 1905; *A Theory of Protein Metabolism*, 1905; all published in the *American Journal of Physiology*. *Protein Metabolism*

*from the Standpoint of Blood and Tissue Analysis,* with W. Denis, 1912; *The Origin and Significance of the Ammonia in the Portal Blood,* with W. Denis, 1912; *A New Method for the Determination of Hippuric Acid in Urine,* with Fred F. Flanders, 1912; *A New Method for the Determination of Total Nitrogen in Urine,* with Chester J. Farmer, 1912; *New Methods for the Determination of Total Non-protein Nitrogen, Urea and Ammonia in Blood,* with W. Denis, 1912; *Protein Metabolism from the Standpoint of Blood and Tissue Analysis; Further Absorption Experiments with Especial Reference to the Behavior of Creatine and Creatinine and to the Formation of Urea,* with W. Denis, 1912; *Absorption from the Large Intestine,* with W. Denis, 1912; *Absorption from the Stomach,* with Henry Lyman, 1912; *The Uric Acid Problem; An Experimental Study on Animals and Man, including Gouty Subjects,* with Hilding Berglund and Clifford Derick, 1924; all published in the *Journal of Biological Chemistry.*

From the data obtained in the first two papers of the above list, Folin drew some interesting and suggestive generalizations, which led him to the view that the current theories concerning the nature of protein metabolism required reconsideration. The term "normal urine" he deemed a tribute to Voit and his dietary standard of 118 grams of protein food per day. In contrast to the 14-18 grams of total urinary nitrogen, which must be looked on as normal if the Voit and Atwater dietary standards are followed, Folin pointed to his analyses of normal urines which showed a daily total nitrogen varying from 4.2 to 8.0 grams from a man, perfectly normal, who had for

years subsisted on a low nitrogen diet, and 3.8 to 6.5 grams, 3.6 to 6.7 grams and 2.8 to 5.3 grams of urinary nitrogen per day in three other individuals fed on a starch and cream diet. The important matter, however, connected with these figures was the peculiar distribution of the urinary nitrogen. Thus, on a protein-rich diet Folin found 86-89 per cent of the nitrogen was in the form of urea, while on the protein-poor diet the urea-nitrogen had dropped to as low as 62 per cent of the total.

Again, it was observed that where the protein metabolism was reduced toward the minimum, creatinine-nitrogen was markedly increased, in one case even to 11 per cent of the total nitrogen. These and other facts led Folin to the conclusion as a principle in the chemistry of metabolism, that *the distribution of the nitrogen in urine among urea, and the other nitrogenous constituents depends on the absolute amount of total nitrogen present.* Folin deemed the part played by creatinine as a factor in the relative distribution of the urinary nitrogen the most interesting feature of these investigations and he was led to adopt as "another fixed principle in the chemistry of metabolism" the fact that the *absolute quantity of creatinine eliminated in the urine on a meat-free diet is a constant quantity different for different individuals, but wholly independent of quantitative changes in the total amount of nitrogen eliminated.*

This constancy in the absolute quantity of creatinine excreted, while the composition of urine representing an intake of fifteen grams of nitrogen is widely different from the composition of urine representing only three or four grams of nitrogen led Folin to assume the existence of

two forms of protein katabolism, essentially independent and quite different. The one kind, which tends to be constant he would term *tissue* or *endogenous* metabolism, while the other, the variable protein metabolism, he would call *exogenous*. Only a small amount of protein, *i.e.*, that needed for endogenous metabolism is required. "The greater part of the protein furnished with standard diets like Voit's is not needed, or to be more specific, its nitrogen is not needed." It is the endogenous metabolism that sets a limit to the lowest level of nitrogen equilibrium attainable.

The physiological needs of the body for protein food Folin believed can be met by providing liberally for the endogenous metabolism and for the maintenance of a reasonable supply of reserve protein. The fact, well established, that moderate or even severe muscle work does not increase protein katabolism fits in with Folin's theory that protein katabolism, in so far as its nitrogen is concerned, is independent of the oxidations that give rise to heat or to the energy that is converted into work. "It ought neither to be necessary nor advantageous for the organism to split-off and remove large quantities of nitrogen which it can neither use nor store up as reserve material."

Later, we shall have occasion to refer to other types of experimental work from Folin's laboratory. Finally it may be added as an illustration of the growing importance of metabolism studies in America that in 1922 there was established *The Journal of Metabolic Research,* especially devoted to original research in metabolism, particularly in pathological conditions.

# CHAPTER IV

Study of Proteins—The work of Thomas B. Osborne, Osborne and Mendel—Amino-acids in relation to growth—William C. Rose, the nutritive importance of certain protein derivatives—Henry D. Dakin, the amino-acids of gelatin—Donald D. Van Slyke, the fate of protein digestion products in the body—The proteans of Osborne—Relation of proteins to acids and bases, studies by Lawrence J. Henderson—Jacques Loeb, the chemical and physical behavior of proteins—Studies of Edwin J. Cohn and collaborators on the isoelectric points of certain proteins—Lucius L. Van Slyke, casein and caseinates—Studies on nucleoproteins, Walter Jones and associates—Nuclease and kindred enzymes—Nucleic acid and its derivatives, Phoebus A. Levene and collaborators—Triticonucleic acid, Thomas B. Osborne and I. F. Harris—Synthetical studies of pyrimidines, Henry L. Wheeler and Treat B. Johnson—Tubercle nucleic acid, Treat B. Johnson and Elmer B. Brown—Murexide, Slimmer and Stieglitz.

The story of the gradual development of knowledge regarding proteins, the chemical basis of all animal and vegetable tissues, constitutes one of the most interesting chapters in the history of physiological chemistry. As the mother substances from which come a great variety of cleavage products, all more or less conspicuous as metabolites in the physiological processes of the animal body, they occupy a position of peculiar significance. At one time, as is well known, protein was looked upon as a single substance (Mulder, Liebig), such differences in behavior as were observed in the protein obtained from

various sources being ascribed to changes in physical conditions. Gradually, however, through the work of many investigators there came recognition of divergent forms of natural proteins, fifty or more in number, divided into various groups on the basis of origin, solubility, precipitability, coagulability, and other physical properties.

Today, we recognize a very large number of more or less closely related proteins, whose superficial differences are of far less significance than the differences in their chemical structure. Obviously, the chemical make-up of the protein molecules is a matter of the greatest importance chemically and physiologically, for unless there is definite knowledge of the chemical constitution and physico-chemical behavior of these all-important substances there can be no clear understanding of the intricacies of protein metabolism. By the application of methods of oxidation, and especially of hydrolysis and cleavage, the presence of certain amino-acids was detected, so that even in Liebig's time, or shortly thereafter, it was recognized that the protein molecule contained a number of such compounds, of which, however, only a few had been separated.

In more recent years, thanks especially to the labors of Drechsel, Kossel, Hopkins, Hofmeister, Abderhalden, and above all, to the epoch-making discoveries of Emil Fischer, a long list of mono-amino and diamino-acids have been identified, and adequate methods developed for determining the amounts formed in the hydrolysis of different proteins. From knowledge so gained, has come the belief that these amino-acids are the true foundation stones or units of which the protein molecule is constructed. As Hofmeis-

ter has pointed out, proteins are probably built up by the condensation of several amino-acids, thereby forming a class of products which have been termed *polypeptides* by Fischer, the most probable mode of union of the amino-acids being a combination through a nitrogen atom. These *polypeptides,* of which there can be many, varying with the amino-acids present, undoubtedly make up the nucleus or the essential part of the structure of the protein molecule, to which may be added other groups to build the finished product.

While credit must be given very largely to the chemists of Germany for the knowledge that has been acquired regarding the nature of proteins, the chemists of America have contributed much to confirm and enlarge understanding of this vital subject. Among such workers Thomas B. Osborne stands out as the most conspicuous, judged from his accomplishments in this field.

Thomas Burr Osborne was trained in chemistry at Yale, working for a time in the Sheffield Laboratory of Physiological Chemistry. From 1886 to 1928 he held the position of research chemist at the Connecticut Agricultural Experiment Station. He was likewise research associate in biochemistry at Yale and research associate of the Carnegie Institution of Washington. His life has been devoted almost entirely to research work in protein chemistry, especially proteins of vegetable origin, his interest in this subject having been stimulated by Professor Samuel W. Johnson, under whose direction he undertook his first investigations. In his earlier work his activities were directed mainly to the preparation of pure products from various sources, with a study of their more striking char-

acteristics, composition, the denaturing action of acids, alkalies and metallic salts, hydrolysis by acids and alkalies, partition of nitrogen, ratio of sulfide sulfur to total sulfur, etc., devoting special attention to establishing constant differences between the proteins of different plants.

Among his numerous publications, numbering more than one hundred, may be mentioned: *The Proteids or Albuminoids of the Oat Kernel*, 1891; *A Study of the Proteids of the Corn or Maize Kernel*, with R. H. Chittenden, 1891; *Crystallized Vegetable Proteids*, 1892; *The Proteids of Barley*, 1895; *Proteids of the Pea*, with G. F. Campbell, 1898; *The Proteins of the Wheat Kernel*, 1907; *Hydrolysis of Legumin from the Pea*, with S. H. Clapp, 1907; *Hydrolysis of Vetch Legumin*, with F. W. Heyl, 1908; *Analysis of the Products of Hydrolysis of Wheat Gliadin*, with H. H. Guest, 1911; *Nitrogen in Protein Bodies*, with I. F. Harris, 1903; *The Proportion of Glutaminic Acid Yielded by Various Vegetable Proteins when Decomposed by Boiling with Hydrochloric Acid*, with R. D. Gilbert, 1906; *A New Decomposition Product of Gliadin*, with S. H. Clapp, 1907; *The Different Forms of Nitrogen in Proteins*, with C. S. Leavenworth and C. A. Brautlecht, 1908; *Some Modifications of the Method in Use for Determining the Quantity of Mono-amino-acids Yielded by Proteins when Hydrolyzed with Acids*, with D. B. Jones, 1910; *Do Gliadin and Zein Yield Lysine on Hydrolysis?*, with C. S. Leavenworth, 1913; *Does Gliadin Contain Amide Nitrogen?*, with O. L. Nolan, 1920.

Osborne's earlier work showed clearly the great difficulty in establishing the strict chemical individuality of

the vegetable proteins. As he stated, "the best that can be done at present is to establish a constancy of the ultimate composition of successive fractional precipitations of the protein under consideration, and to show the constancy of the physical properties and products of hydrolysis of these fractions so far as this is possible." He, however, determined the chemical composition of a great variety of vegetable proteins, products of reasonably definite character, crystalline and others. Of special interest were the studies carried out by Wells and Osborne of the anaphylaxis reaction as a means of establishing chemical identity, the more important papers on this subject by H. G. Wells,[1] and T. B. Osborne, published in the *Journal of Infectious Diseases*, being the following: *The Biological Reactions of the Vegetable Proteins*, 1911; *Is the Specificity of the Anaphylaxis Reaction Dependent on the Chemical Construction of the Proteins or on their Biological Relations?* 1913; *The Anaphylactogenic Activity of Some Vegetable Proteins*, 1914; *The Anaphylactic Reaction with So-called Proteoses of Various Seeds*, 1915; *Anaphylaxis Reactions between Proteins from Seeds of Different Genera of Plants*, 1916; *Anaphylaxis Reactions with Purified Proteins from Milk*, 1921.

Extensive comparisons of numerous proteins tested as to their anaphylaxis reaction showed "that each seed contains several chemically distinct proteins, and that no two seeds, unless very closely related botanically, contain chemically identical proteins." By this method of

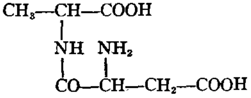
[1] H. Gideon Wells, professor of pathology in the University of Chicago and director of medical research Otho S. A. Sprague Memorial Institute, Chicago. He was a student in the Sheffield Laboratory of Physiological Chemistry at Yale in 1894, and did research work there in later years.

testing it became evident, as Osborne has stated, that there must be an almost infinite variety of vegetable proteins, the anaphylactogenic property of a protein being determined by its chemical structure and not by its biological origin.[2]

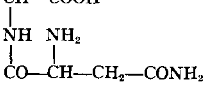
Among the vegetable proteins, the alcohol-soluble products, of which zein is a type, are especially worthy of note. Osborne proposed the name of "prolamins" for these proteins, since they all yield on hydrolysis a relatively large quantity of both proline and amide nitrogen. As to whether the proteoses which can be separated from seeds are present as such, or are formed during the processes of extraction and separation, seems uncertain. Wells and Osborne found, however, that such proteoses were highly anaphylactogenic, whereas the proteoses formed by the artificial digestion of vegetable proteins with pepsin or trypsin were without such action. In this connection attention may be called to a number of investigations carried on in the Sheffield Laboratory of Physiological Chemistry, relating to the formation of proteoses and peptones by the digestion of vegetable proteins, *viz.: Crystalline Globulins and Globuloses or Vitelloses,* by R. H. Chittenden and J. A. Hartwell, 1890; *On the Primary Cleavage Products Formed in the Digestion of Gluten-Casein of Wheat by Pepsin-Hydrochloric Acid,* by R. H. Chittenden and E. E. Smith, 1890; *On the Proteolysis of Crystallized Globulin,* by R. H. Chittenden and L. B. Mendel, 1894, all published in the English *Journal of Physiology.*

[2] For further consideration of anaphylaxis and the work of Wells, see Chapter VIII.

Regarding the partition of nitrogen in the protein molecule, especially in vegetable proteins, Osborne and his associates, using the Hausmann method with some modifications, hydrolyzed thirty-seven individual proteins, representing various types from different sources. The most striking feature of their results was the wide range in the amount of basic nitrogen obtained, *viz.*, from one-fourth to one-thirtieth of the total nitrogen of the protein, while the proportion of ammonia differed from one-fourth to one-sixteenth of the total nitrogen. The non-basic nitrogen, on the other hand, was found to be more constant than the total nitrogen and formed about one-half to three-fourths of the latter. As to the ammonia which results from protein hydrolysis, Osborne's experimental data suggested very strongly that the nitrogen yielding this ammonia was in amide combination with one of the carboxyl groups of the dibasic amino-acids. In other words, the ammonia comes from the hydrolysis of $CONH_2$ groups with formation of COOH groups; a view supported by the fact that on hydrolysis of gliadin the acidity of the products of hydrolysis increased in proportion to the ammonia set free. If the amino-acids are combined in the protein molecule in peptide union as in the peptide of alanine and aspartic acid, the dibasic acids could furnish carboxyl groups, with which nitrogen might combine in amide union, as indicated in the accompanying formulas:




*peptide*




*amide of the peptide*

Experiments by Osborne and his co-workers showed that a large proportion of glutaminic acid is in many cases accompanied by a similar large proportion of amide nitrogen, thus supporting the view that a relation may exist between the amount of amide nitrogen which different proteins yield and the amounts of glutaminic and aspartic acids present in the molecule. As Osborne has stated, experiments made in his laboratory (1908) showed that "the amount of ammonia yielded by hydrolysis with acids, agreed so closely with that required for amide union with the sum of the glutaminic and aspartic acids found in a large number of proteins of both vegetable and animal origin as to make it highly probable that practically all of the ammonia originated from such a combination and that one of the carboxyl groups from each molecule of the dibasic acids was thus united with a $NH_2$ group."

It is the character and amounts of the various substances represented by the basic and non-basic nitrogen of the protein molecule, however, that have greatest interest, since they throw light on radical points of difference of both chemical and physiological significance. The identification and separation of the amino-acids formed in the hydrolysis of proteins, was an exceedingly difficult problem, but by 1907, Osborne had accomplished hydrolysis of the proteins of the wheat kernel[3] with the following results:

The inadequacy of the methods available for separating the various amino-acids at the time these studies were

[3] "The Proteins of the Wheat Kernel," by Thomas B. Osborne, Carnegie Institution of Washington, Publication No. 84.

| | *Leucosin* | *Gliadin* | *Glutenin* |
|---|---|---|---|
| Glycocoll | 0.94 per cent | 0.00 per cent | 0.89 per cent |
| Alanine | 4.45 | 2.00 | 4.65 |
| Amino-valerianic acid | 0.18 | 0.21 | 0.24 |
| Leucine | 11.34 | 5.61 | 5.95 |
| α-proline | 3.18 | 7.06 | 4.23 |
| Phenylalanine | 3.83 | 2.35 | 1.97 |
| Aspartic acid | 3.35 | 0.58 | 0.91 |
| Glutaminic acid | 6.73 | 37.33 | 23.42 |
| Tyrosine | 3.34 | 1.20 | 4.25 |
| Lysine | 2.75 | 0.00 | 1.92 |
| Histidine | 2.83 | 0.58 | 1.76 |
| Arginine | 5.94 | 3.16 | 4.72 |
| Ammonia | 1.41 | 5.11 | 4.01 |
| Tryptophane | present | present | present |
| Serine | .... | 0.13 | 0.74 |
| Cystine | .... | 0.45 | 0.02 |
| | 50.32 | 65.78 | 59.66 |

made, is clearly suggested by the incompleteness of the above analyses, for there was no good ground for believing that these proteins contained any quantity at least of other amino-acids, either acidic, basic, or neutral. Dakin, however, discovered in 1918, oxyglutaminic acid, the amount in zein being 2.5 per cent, but this would not raise the percentages very largely. Of more significance were the results obtained by D. Breese Jones and Otto Moeller (1928), in the laboratory of the *Protein and Nutrition Division, Bureau of Chemistry and Soils,* United States Department of Agriculture, where by the use of new and better methods of analysis, they showed the percentages of aspartic and glutaminic acids obtainable from such proteins as gliadin and edestin to be much

higher than heretofore reported. Further, as Osborne and Vickery have recently pointed out, in *Physiological Reviews*,[4] 1928, zein is the most completely analyzed protein up to this time, and adding together the highest percentage results obtained by Osborne and Liddle, Dakin, Looney, Kossel and Kutscher in their determinations of the various amino-acids formed by hydrolysis of this protein, the total rises to 102.56 per cent.

Disregarding the question of completeness in Osborne's data bearing on the three proteins of the wheat kernel, the figures given afford a striking illustration of the great difference in the organic make-up of proteins from the same seed. Moreover, what is seen here is found to be characteristic of all proteins of both vegetable and animal origin, testifying to the great diversity of their individual chemical structure. In twenty-six proteins, all but four being vegetable in origin, Osborne and his co-workers found the following variations in the content of the basic amino-acids: histidine 0.39 per cent in gliadin from rye to 3.08 per cent in vignin from the cow pea; arginine 1.55 per cent in zein to 14.44 per cent in the globulin from squash seed; lysine 0 in zein to 6.43 per cent in conalbumin from hen's egg. Especially interesting was the discovery of a dipeptide of proline and phenylalanine separated by Osborne and Clapp from the products formed in the hydrolysis of gliadin; a beautifully crystalline compound which on hydrolysis with a strong acid yielded proline and phenylalanine in molecular proportions.

[4] "A Review of Hypotheses of the Structure of Proteins," by Hubert Bradford Vickery and Thomas Burr Osborne. *Physiol. Rev.* 8: (1928), edited by the American Physiological Society.

While the differences in the chemical constitution of proteins indicated by the preceding statements have great chemical significance, they likewise suggest that the individual proteins must have different nutritive values. As is well known, however, it is not the protein itself that is absorbed making a direct contribution to the metabolism of the body, but it is the amino-acids set free by the digestion of the protein in the gastro-intestinal tract that are the essential factors in maintenance and growth. To be sure, under ordinary conditions of life, people naturally consume mixtures of foodstuffs, where deficiencies in one particular protein may be made good by a surplus of certain amino-acids, for example, present in the other proteins eaten. But as a physiological problem it is important to know whether a given protein is alone adequate to maintain an animal in normal condition, and also whether it is capable of promoting growth at a normal rate. In other words, the true nutritive value of the individual proteins must be ascertained so far as possible.

Thus, as Osborne and his associates have found, zein, which constitutes nearly one-half of the maize kernel, is lacking in glycocoll, tryptophane, lysine and oxyproline (Dakin). Rats of different ages, as Osborne and Mendel found, when fed on diets in which about fifteen per cent of their calories were in the form of zein rapidly declined and died. If, however, about 0.5 per cent of tryptophane was added to the diet, body-weight was maintained, but there was no growth unless a small amount of lysine was also added. The physiologist, therefore, must discriminate between "complete" and "incomplete" proteins, between those of good or poor "biological quality."

Today in studying the problems of nutrition it is recognized that several of the amino-acids derived from the proteins of the food are imperatively needed for the building of tissue and for making good the losses of cellular material. As Mendel has stated, "the efficiency of the individual protein in this respect must depend on the minimum of any indispensable amino-acid that it will yield; for it is now known that some of them cannot be synthesized anew by the animal organism."

The nutritive value of the vegetable proteins has been made the subject of a long and productive series of studies by Osborne and Mendel dating from 1911, carried on at the Connecticut Agricultural Experiment Station and the Sheffield Laboratory of Physiological Chemistry at Yale, and supported in part by the Carnegie Institution of Washington. Lafayette B. Mendel, a graduate of Yale College, 1891, took up the study of physiological chemistry in the Sheffield Scientific School immediately after his graduation, acquiring in due time the Ph.D. degree. He then served as instructor, assistant professor, and since 1903 as professor of physiological chemistry, in the Sheffield Scientific School. In 1921, he became the Sterling professor of physiological chemistry in the University. He is also a research associate of the Carnegie Institution of Washington. During one year, he carried on research at the universities of Breslau and Freiburg.

Of the many publications by Osborne and Mendel of the results of their work, the following may be cited: *The Rôle of Gliadin in Nutrition*, 1912; *Amino-acids in Nutrition and Growth*, 1914; *The Comparative Nutritive Value of Certain Proteins in Growth and the Problem of the*

*Protein Minimum*, 1915; *The Amino-acid Minimum for Maintenance and Growth as Exemplified by Further Experiments with Lysine and Tryptophane*, 1916; *The Protein Factor in the Seeds of Cereals*, 1918; *The Nutritive Value of the Wheat Kernel and its Milling Products*, 1919; *Nutritive Value of the Proteins of the Barley, Oat, Rye, and Wheat Kernels*, 1920; all published in the *Journal of Biological Chemistry*.[5]

It is not possible to discuss here in detail the character of their many findings, but the results of their feeding experiments with white rats constitute a substantial contribution to knowledge of the nutritive deficiencies of those proteins in which there is a shortage or a complete absence of certain amino-acids, such as lysine, cystine and tryptophane. These three amino-acids unquestionably are necessary for growth, as the accumulated experience of many workers prove. As to arginine and histidine the evidence has not been so clear. Animals fed on "inferior" protein quickly show the effects of a diet which fails to supply in sufficient amount the amino-acids needed for proper growth. The work of Osborne and Mendel has done much to establish the close relationship between the chemical constitution of proteins and their biological value in nutrition. In 1916, in the *Ergebnisse der Physiologie*, under the title "Das Wachstum," Mendel wrote a comprehensive review of the broad subject of growth, with inclusion of all the chemical data then available.

For some years, mainly as the result of studies by

[5] A valuable summary of all Osborne's work on the vegetable proteins is to be found in one of the monographs on biochemistry, edited by Plimmer and Hopkins, Osborne, Thomas B., "The Vegetable Proteins," 1924.

Ackroyd and Hopkins, and by Abderhalden, it was considered that arginine and histidine, one or both, since it was deemed probable they were inter-convertible in the animal organism, were essential for growth. A number of investigations, carried on at the University of Illinois, by William C. Rose and his co-workers have, however, thrown much light on this matter.

William Cumming Rose had his training in physiological chemistry in the Sheffield Scientific School at Yale, taking the Ph.D. degree in 1911, and serving there for a time as assistant in physiological chemistry. As professor of physiological chemistry at the University of Illinois he has accomplished work of great physiological importance. The papers which have interest in the present connection are *The Relation of Arginine and Histidine to Growth,* with Gerald J. Cox, 1922; *The Relation of Histidine and Arginine to Creatine and Purin Metabolism,* with Kenneth G. Cook, 1925; *Can Purins, Creatinine or Creatine Replace Histidine in the Diet for Purposes of Growth?,* with Gerald J. Cox, 1926; *The Availability of Synthetic Imidazoles in Supplementary Diets Deficient in Histidine,* with Gerald J. Cox, 1926; *Growth upon Diets Practically Devoid of Arginine, with Some Observations upon the Relation of Glutamic and Aspartic Acids to Nutrition,* with W. Edward Bunney, 1927; all published in the *Journal of Biological Chemistry.*

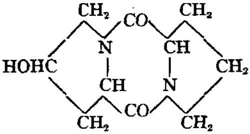
Rose and his associates found that rats could be maintained for long periods of time, with excellent growth, upon diets practically free from arginine; that arginine was quite incapable of improving the quality of a diet deficient in histidine; that in the absence of arginine the inclusion of histidine in the ration leads to rapid growth.

Consequently, it would appear that histidine is an indispensable amino-acid, while arginine is apparently not necessary for normal nutrition. Further, histidine and arginine are not interchangeable in metabolism.

In view of the nutritive importance of histidine it became desirable to ascertain whether other substances having related structural configuration, such for example as creatinine, a derivative of the glycocyamidine ring or the purins, such as adenine and guanine, derivatives of the imidazole ring, can take the place of this amino-acid in nutrition, *i.e.*, whether the animal organism can transform any of these substances into histidine. The work of Rose and his co-workers has tended to corroborate the conclusion of Hopkins and Ackroyd that histidine is one of the precursors of purins, probably the mother substance of allantoin. Feeding experiments, however, with growing animals, using diets deficient in histidine, but adequate in every other respect, showed that neither adenine, guanine, creatine, creatinine, nor a combination of these, can take the place of the missing amino-acid. It is thus apparent that the reaction of purin synthesis from histidine is an irreversible one in the animal organism. On the other hand, the addition of *dl*-β-4-imidazole lactic acid to a histidine-deficient diet caused immediate resumption of growth. Hence, it would appear that this synthetic product, presumably by being converted into the amino-acid by the body cells, can take the place of histidine in meeting the nutritional needs of the organism. Finally, it is worthy of note that this constitutes the first successful attempt, in growth experiments, to replace an indispensable amino-acid of the diet by a non-amino compound.

That the amino-acids now recognized as the foundation stones of the various proteins are the sole components of the protein molecule, receives confirmation from the hydrolysis of the scleroprotein, gelatin. Here is a peculiar protein, which might well be expected to have a quite different constitution from that of the proteins of other groups, and in a sense it does have, but the difference is due almost wholly to changes in the proportion of the same amino-acids. Henry D. Dakin, in a paper on *The Amino-acids of Gelatin,* 1920, has recorded his results obtained on analysis of the products formed by the hydrolysis of gelatin, the total of amino-acids actually determined being 91.31 per cent, "to which must be added considerable amounts of serine and possibly allied substances that could not be separated from the hydroxyproline." In his analysis of these amino-acids from gelatin, several were reported in higher proportion than heretofore recorded, *viz.*, glycocoll, 25.5 per cent; alanine, 8.7 per cent; aspartic acid, 3.4 per cent; histidine, on the other hand, only 0.9 per cent.

Especially noteworthy was the discovery by Dakin of a new tricyclic peptide from gelatin, *viz.*, γ-hydroxyprolylproline anhydride, the first peptide to be obtained containing the hydroxyproline nucleus and unique in containing three rings.



*hydroxyprolylproline anhydride*

Since proteins are broken down more or less completely into amino-acids during the processes of digestion, the question naturally arises as to the steps followed in the utilization of these acids by the organism. Among the many workers in this field America has been well represented by Donald D. Van Slyke, a graduate of the University of Michigan, 1905, Ph.D., 1907, a student at the University of Berlin, 1911. Van Slyke has been since 1907 research chemist at the Rockefeller Institute for Medical Research. In that capacity he has carried on successfully chemical investigations in several fields, notably on the rôle of protein derivatives in physiology under the general title of *The Fate of Protein Digestion Products in the Body.*

His more important papers on this subject are *The Amino-acid Nitrogen of the Blood, Preliminary Experiments on Protein Assimilation,* with Gustav M. Meyer, 1912; *Determination of Amino Nitrogen in the Tissues,* 1913; *The Absorption of Amino-acids from the Blood by the Tissues,* with Gustav M. Meyer; *The Locus of Chemical Transformation of Absorbed Amino-acid,* with Gustav M. Meyer; *The Effects of Feeding and Fasting on the Amino-acid Content of the Tissues,* with Gustav M. Meyer. Emphasis is to be placed upon the fact that Van Slyke in his discovery of a *Method for Quantitative Determination of Aliphatic Amino Groups* gave to physiological chemists not only a method for the direct determination of amino-acids in the blood and tissues, but also one that admitted of broad application, notably in the study of the chemical make-up of protein substances in general and particularly in the study of proteolysis and

proteolytic products. After devising a suitable method for the extraction of the amino-acids from the tissues the amino nitrogen was eventually determined by the nitrous acid method, the figure obtained representing approximately the free $\alpha$-amino-acids.

Among their many results, using dogs as subjects, and amino-acids obtained by the hydrolysis of casein, also free arginine and alanine, it was found that amino-acids injected intravenously disappeared from the circulation rapidly, but never completely; the blood containing 3-8 milligrams of amino-acid nitrogen per 100 cc. even after a fast of several days. This disappearance of the amino-acids from the circulation, Van Slyke believed to be due not to their destruction, synthesis, or chemical incorporation into the cell proteins, but rather to their simple absorption by the tissues, without any immediate chemical change.

Again, on feeding dogs with fresh beef, it was found that five hours after the meal, the amino-acid content of the blood was nearly doubled. This rise in the amino-acid content of the blood during digestion was plainly opposed to the older view that the amino-acids formed in digestion are synthesized, while passing through the intestinal wall, into the blood proteins. Van Slyke's interpretation of his results led to the obvious concept that the amino-acids normally pass the intestinal wall and enter directly into the blood current when, circulating through the entire organism, they are offered to the body cells in general. It was further found that the amino-acids normally never rise beyond a small amount, due to

the fact that the tissues take them up rapidly when they are unusually abundant.

Analysis of the tissues of dogs in various states of nutrition gave, among other results, evidence that the free amino-acids do not disappear from the tissues during fasting; if anything they tend to increase. This was interpreted to mean that autolysis must be the main source of the free amino-acids during fasting. Again, when arginine or alanine was fed to dogs, it was observed that they were excreted within twenty-four hours, in the form of urea, thus indicating that the amino-acids do not remain for long in the organs or tissues of the body; a view which is in harmony with the well-known fact that when protein is fed to a dog in nitrogenous equilibrium there is at once an increased excretion of nitrogen corresponding to the added protein. Apparently, the liver is especially responsible for the katabolism of those digestion products not utilized for tissue construction; *i.e.*, the excess over and above the amount needed for repair. It was further assumed that since each tissue has its own store of amino-acids, which it can replenish from the blood, it uses these to synthesize its own proteins. The amino-acids in the tissues are thus intermediate steps in both the construction and breakdown of the tissue proteins; originating not only from absorbed food products, but also from autolyzed tissue proteins.

That acids and alkalies when added in small quantities to proteins produce changes in solubility has long been known and the terms "acid albumin" and "alkali albumin" or "alkali albuminate" have been in use for many years. Seventy-five years ago Mulder wrote that all forms

of protein have the power of combining with small quantities of bases and acids yielding both insoluble and soluble compounds. The albumin-hydrochloric acid compound, for example, according to Mulder, may contain as much as 3-7 per cent of hydrochloric acid, although it is not probable that it always contains such amounts. Whatever action acids and alkalies may have it is not sufficiently profound to alter the composition or constitution of the protein. This was clearly shown by Osborne (1902) in his experiments with edestin, who stated "that small quantities of acids effect profound changes in the solubility of edestin without altering its ultimate composition sufficiently to be detected by analysis."

Intermediate products between proteins and acid-albumin, however, may be produced by the action of acids. Thus, Osborne found that if crystallized edestin was dissolved in the least possible quantity of hydrochloric acid and then precipitated by addition of a small amount of sodium chloride, the precipitate could not be wholly dissolved in a strong solution of the salt. In other words, a portion of the original edestin had been converted into an insoluble form which could not be made soluble again in a neutral salt solution. Further, as it was not soluble in dilute solutions of potassium hydroxide it could not be acid-albumin. Since practically all seed proteins behave in this manner, Osborne has suggested for such insoluble products the general term "proteans."

Especially interesting was the observation made by Osborne that the acidity of edestan chloride was three times that of edestin chloride; *i.e.*, that the basic property of this altered product was much greater than that of the

protein from which it was formed. Toward alkalies the vegetable proteins are more resistant. Thus, Chittenden and Osborne (1891) found that zein, even when exposed to the action of a two per cent solution of potassium hydroxide at 40° C., for twenty-four hours, did not lose its original solubility in alcohol, although it is possible an "alkali albumin" soluble in alcohol may have been formed.

Development of the modern theories of ionization has naturally introduced a new and important factor in the study of the relation of proteins to acids and bases. To this and allied studies America has contributed much and the names of Lawrence J. Henderson, Jacques Loeb, and L. L. Van Slyke stand out conspicuously as workers in this field.

Lawrence J. Henderson, a graduate of Harvard University, A.B., 1898, M.D., 1902, a worker at the University of Strassburg 1902-1904, has been since 1904 lecturer, instructor, assistant professor and professor of biological chemistry (1919) at Harvard. His research work has been directed especially to the applications of physical chemistry to biological problems. In 1909 there was published by Henderson in the *Ergebnisse der Physiologie,* Band 8, under the title *Das Gleichgewicht zwischen Basen und Säuren im Thierischen Organismus,* a detailed account of his studies covering such subjects as regulation of neutrality, theories of solutions, ionization constants, equilibrium in blood and blood plasma, influence of temperature and other phases on equilibrium, etc., with special reference to physiological conditions, together with a general discussion of the various views held at that date. Further, in Osborne's *The Vegetable Pro-*

*teins,* 1924,[6] there is a chapter by Henderson dealing especially with the *Relation of Proteins to Acids and Bases.*

It is not possible here to enter on any detailed discussion of methods, or the different lines of reasoning employed; it must suffice to state a few general conclusions. Protein may be looked upon as an amphoteric electrolyte, which through ionization can form both hydrogen- and hydroxyl-ions.

$$H \cdot R \cdot OH = \overset{+}{H} + \overset{+}{R} + \overline{OH}$$

Thus the ionization of a simple protein derivative like glycocoll can be assumed to take place as follows:

$$\begin{matrix} CH_2 \cdot NH_3\ OH \\ | \\ COOH \end{matrix} = \begin{matrix} CH_2\ \overset{+}{NH_3} \\ | \\ CO\overline{O} \end{matrix} + \overset{+}{H} + \overline{OH}$$

In their relations with acids and bases Henderson found that "proteins behave as if there were present in the molecule a considerable number of acid radicals of various degrees of strength and a considerable number of similar basic radicals"; a conclusion in harmony with present-day theories regarding the constitution of the protein molecule. When the ionization of a protein through its acid radicals and that through its basic radicals are equal (the isoelectric point), the solubility of the protein may be assumed to be at the minimum, "since it is probably the undissociated molecule which chiefly determines solubility, the ions being relatively soluble substances."

[6] See Reference 5.

Again, as Henderson has stated, "the isoelectric point or zone may fall at an acid, neutral or alkaline reaction, according to the relative number and strength of the acid or basic groups of the molecule. Thus, if the acid groups be numerous and strong, and the basic groups less numerous and weak, it is evident that the isoelectric state must occur at an acid reaction. Moreover, in case both acid and basic radicals are all weak, there will be a wide zone of reaction throughout which the protein will be very slightly combined with either acid or base, and within which, as a first approximation, it may be regarded as free from such combination." Consequently, vegetable proteins being essentially free from salt formation near their isoelectric points, they can, while in that state, be prepared as pure substances. Finally it would seem that proteins, in spite of their great complexity of structure and the great size of their molecules, form salts with both acids and bases in harmony with ordinary chemical laws; "definite salts containing acid and protein in true stoichiometrical ratios."

In opposition to these views, the belief has been more or less current, that proteins being colloidal in character, must follow the rules of "adsorption" in their reactions with other bodies; that the chemistry of colloids must differ from the chemistry of crystalloids. This view has been vigorously combated by Jacques Loeb, whose many investigations of amphoteric colloids have given a fund of information regarding the chemical and physical behavior of proteins.

Jacques Loeb, educated at the universities of Berlin and Strassburg, was on the faculty of the University

of Chicago from 1892-1902 and at the University of California 1902-1910, holding the chair of physiology and experimental biology. In 1910 he became the head of the department of experimental biology at the Rockefeller Institute for Medical Research, which position he held until his death in 1924. His researches in general physiology, dynamics of living matter, artificial parthenogenesis, chemical stimulation of development, etc., are known and recognized throughout the scientific world. He was one of the two editors of the *Journal of General Physiology,* established in 1918.

Loeb's experimental work on colloids, that may be noted in this connection, is covered in part by the following papers: *Volumetric Analysis of Ion-Protein Compounds; The Significance of the Isoelectric Point for the Purification of Amphoteric Colloids,* 1918; *Ion Series and the Physical Properties of Proteins,* 1920, covering such topics as the *Action of Weak and Strong Monobasic Acids, Combining Ratios of Acids and Alkalies with Crystalline Egg Albumin and the Osmotic Pressure of the Albumin Solutions, Combining Ratios of Acids and Bases with Gelatin and the Viscosity of Gelatin Salts; The Action of Salts in Low Concentration,* 1920; *Chemical and Physical Behavior of Casein Solutions,* 1921; *The Colloidal Behavior of Proteins,* 1921; all published in the *Journal of General Physiology.*

The lack of experimental evidence that ions unite with proteins in the typical ratio in which the same ions combine with crystalloids had long been the stumbling-block that prevented chemists from accepting whole-heartedly the view that the forces by which proteins combine with

acids and alkalies are the chemical forces of primary valency. Loeb's work, however, led to the necessary proof, when he found "that the hydrogen-ion concentration of the protein solution determines the amount of ion entering into combination with a protein, and that therefore the ratios in which different ions combine with proteins must be compared for the same hydrogen-ion concentration." Employing protein solutions not only of known concentration of protein but of the same hydrogen-ion concentration as the standard of comparison, he found that acids, alkalies and neutral salts combined with proteins in the same ratios with which they combine with crystalloids. Further, he stated that the influence of the different ions upon the physical properties of proteins can be predicted from the general combining ratios of these ions.

Contrary to the older view that both ions of a neutral salt *are adsorbed simultaneously by non-ionized protein* molecules, Loeb was able to show that only the cation or only the anion or that neither can combine at one time with a protein, dependent solely upon the hydrogen-ion concentration of the solution. As defined by their hydrogen-ion concentration proteins exist in three states, as non-ionogenic or isoelectric protein, metal proteinate, and protein acid salts. Finally, comparing the physical properties of solutions of proteins having the same hydrogen-ion concentration Loeb was led to the conclusion that "all acids whose anion combines as a monovalent ion raise the osmotic pressure, viscosity, swelling of protein about twice as much as the acids whose anion combines

as a bivalent anion for the same pH. The same valency rule holds for the cations of different alkalies."

From the Harriman Research Laboratory of Roosevelt Hospital, New York, came in 1919 an interesting paper by Edwin J. Cohn, Joseph Gross and Omer C. Johnson on *The Isoelectric Points of the Proteins in Certain Vegetable Juices*,[7] in which it was pointed out that knowing the isoelectric point of a protein, the nature of the compound of the protein that exists at any hydrogen-ion concentration can be ascertained. The experimental work was carried out on the juices of the potato, carrot and tomato, with a view to ascertaining the character of the compound of the protein as it existed in Nature. As the authors stated, the condition "in which a protein substance exists depends upon the nature of its combination with acids or bases and is changed by change in the protein compound." By adding acid to potato juice the tuberin compound present was dissociated and the tuberin was liberated at its isoelectric point. Since 1922 Cohn has been assistant professor of physiological chemistry at the Harvard Medical School where he has been engaged in outstanding studies on the molecular weights and certain physico-chemical properties of proteins.

Another worker in this field whose researches have gained wide recognition is Lucius L. Van Slyke, who, since 1890, has been the chief chemist of the New York State Agricultural Experiment Station at Geneva and also professor of dairy chemistry at the New York State College of Agriculture since 1920. Of his more important studies with casein, carried out with Alfred W. Bosworth, the fol-

[7] *J. Gen. Physiol.*, 2: (1919).

lowing may be mentioned: *Preparation and Composition of Basic Calcium Casein and Paracaseinate,* 1913; *Preparation and Composition of Unsaturated or Acid Caseinates and Paracaseinates; Valency of Molecules and Molecular Weights of Casein and Paracasein,* published in the *Journal of Biological Chemistry.*

Their results have contributed largely to the view that proteins follow ordinary chemical laws in combining with acids and bases. A few of their findings may be stated here. The monobasic caseinates and paracaseinates while insoluble in water are dissolved by warm 5 per cent solutions of sodium chloride, ammonium chloride, potassium chloride, etc., the solubility being due to an exchange of bases. Thus, monocalcium caseinate treated with sodium chloride reacts with formation of the soluble sodium caseinate and calcium chloride, the reaction being reversible. In the soluble dibasic caseinates 1 gram of casein combines with 2.25 gram equivalents expressed as hydroxide, while in the insoluble monobasic caseinates 1 gram of casein combines with $1.125 \times 10^{-4}$ gram equivalents expressed as hydroxide. In the paracaseinates, twice the amount of base combines with the protein molecule, *i.e.,* 1 gram paracasein combines with 4.50 gram equivalents expressed as hydroxide in the dibasic compounds and with 2.35 in the monobasic. On the basis of their many analytical results, obtained with these casein compounds, Van Slyke and Bosworth concluded that the molecular weight of casein is 8,888, while that of paracasein is 4,444. The valency of the protein molecule in basic caseinates is 8, in basic paracaseinates 4.

Passing to the group of conjugated proteins we are

brought face to face with the nucleoproteins, the most important and at the same time the most significant of the compound proteins; significant because of the complexity of the nucleic acid which combined with albumins, histones or protamines make up the nucleoprotein compounds. In a sense, nucleoproteins, as prepared from aqueous gland extracts, are theoretically salts of protein with nucleic acid in which the protein is in large excess. This excess of protein, however, can be easily removed by digestion with pepsin-hydrochloric acid, when a more resistant mixture of acid salts results, *i.e.*, nuclein. In reality, "nucleoproteins" as prepared in the laboratory are undoubtedly mixtures of various salts of protein with nucleic acid, in which the protein is in excess with admixture of more or less impurities. As Walter Jones has expressed it, "nucleoprotein means rather a method of preparation than a chemical substance."

While nucleoproteins and nucleins are salts of protein with nucleic acid, and undoubtedly exist as such in cell protoplasm and in cell nuclei, it is the nucleic acid which has for both the physiologist and the chemist the greatest interest. Nucleic acids, as is well known, are polybasic acids and proteins are polyacid bases, consequently a large number of salts of the two are possible. Many years ago, 1874, Miescher found the spermatozoa heads of the salmon to be composed almost entirely of a single chemical substance, protamine united to nucleic acid, *i.e.*, protamine nucleate, the simplest nucleoprotein or nuclein known. In tissue and gland cells, however, it is easy to recognize the possible presence of many divergent forms of nucleins and nucleoproteins, made up of mixtures vari-

ously combined, in all of which, however, some form of nucleic acid is a conspicuous feature.

However many nucleic acids there may be, representatives of different gland cells and different plant tissues, they all have apparently the same type of chemical structure. To Kossel, who was the first to study the hydrolytic products of nucleic acid (thymus nucleic acid), we owe in large measure our understanding of the nature of this substance. In the words of Walter Jones, "animal nucleic acid is a dehydrolyzed product of phosphoric acid, hexose and four nitrogenous ring compounds, guanine, adenine, cytosine and thymine. These six substances constitute the fundamental groups of nucleic acid; they stand for nucleic acid."

Naturally, the importance and wide-spread distribution of nucleoproteins and nucleins, the singular and suggestive make-up of the nucleic acid molecule with its contained phosphoric acid, a carbohydrate group together with a purine and a pyrimidine group, attracted the efforts of many workers in organic chemistry. In no less degree were physiologists interested, since the presence of purine derivatives, linked up as they are with other products of the metabolic processes of the animal body, called for a clearer understanding of chemical and genetic relationships. In the gaining of this knowledge many American workers have contributed largely, notably Walter Jones and his associates, Phoebus A. Levene and his coworkers, Thomas B. Osborne, Henry L. Wheeler and Treat B. Johnson.

Walter Jones has been identified with The Johns Hopkins University since 1895, at first as assistant and asso-

ciate, and in 1908 as professor of physiological chemistry. In his laboratory at Baltimore a large amount of experimental work bearing on nucleic acid and its derivatives has been carried on since 1899, with results of great value. He and Levene have been the two outstanding workers in this field in America.

The following papers by Jones may be cited as illustrating the character of his researches: *Über das Thymin,* 1899; *Über das Enzym der Thymusdrüse,* 1904; *Über die Selbstverdauung der Nucleoproteiden,* 1904; *Über die Guanase,* with C. L. Partride, 1904; *Über die Adenase,* with M. C. Winternitz, 1905; *Über die Beziehung der aus wässerigen Organextracten gewonnen Nucleinfermente zu den physiologischen Vorgängen im lebenden Organismus,* 1910; all published in the *Zeitschrift für physiologische chemie; On the Identity of the Nucleic Acids of the Thymus, Spleen and Pancreas,* 1908; *Concerning Nucleases,* 1911; *On the Formation of Guanylic Acid from Yeast Nucleic Acid,* 1912; *On the Guanylic Acid of the Spleen,* with L. G. Rowntree; *The Partial Enzymatic Decomposition of Yeast Nucleic Acid,* with A. E. Richards, 1914; *The Mode of Nucleotide Linkage in Yeast Nucleic Acid,* with B. E. Read, 1917; *Adenine-uracil Dinucleotide and the Structure of Yeast Nucleic Acid,* with B. E. Read, 1916; *Uracil-cytosine Dinucleotide,* with B. E. Read, 1917; *The Structure of the Purine Mononucleotides,* with B. E. Read, 1917; all published in the *Journal of Biological Chemistry.*

Mention should also be made of the monograph written by Walter Jones, entitled *Nucleic Acids, Their Chemical Properties and Physiological Conduct,* 1914, one of the

Monographs on Biochemistry edited by Plimmer and Hopkins. The large volume of experimental work by Jones and his associates does not admit of any adequate presentation or detailed analysis here, but it constitutes a striking illustration of the vigorous growth of biochemical activity which has taken place in this country during recent years.

From the viewpoint of physiology, the two aminopurine derivatives of nucleic acid, guanine and adenine, have special significance, since they stand closely related to the three oxypurines, hypoxanthine, xanthine and uric acid, all five being chemical derivatives of the same mother substance, purine. As is well known, the aminopurines are convertible into the oxypurines by hydrolytic and other agencies, guanine into xanthine, adenine into hypoxanthine, while by reduction xanthine and hypoxanthine can be formed from uric acid; very suggestive reactions from the viewpoint of the metabolic processes of the body.

Again, the pyrimidine derivatives of nucleic acid, cytosine, thymine and uracil, offer another group of special physiological significance; bodies which correspond to pyrimidine groups present as such in nucleic acid, although at one time it was thought they might possibly be formed from the purine groups, a view not now accepted. In this connection it is to be remembered that while cytosine is produced by hydrolysis from the nucleic acid of both animal and plant origin, thymine comes only from animal nucleic acid and uracil only from nucleic acid of vegetable origin. The work of Jones with thymine helped to make clear the existence in the molecule of an

alloxan ring with formation of urea. The structure of all three pyrimidines suggests the ease with which urea may result from such antecedents by the processes of metabolism.

Especially important was the decomposition of yeast nucleic acid into two dinucleotides (nucleotides being compounds in which a carbohydrate group links a phosphoric acid group with a pyrimidine or purine group), one of which yielded guanine and cytosine but no adenine nor uracil, while the other yielded adenine and uracil but no cytosine and at most only a trace of guanine, due presumably to a slight admixture of the other dinucleotide.

Jones' experimental work with B. E. Read led him to the belief that the nucleotide groups of yeast nucleic acid are joined together through their carbohydrate groups, giving rise to a polysaccharide structure. Likewise in his study of adenine-uracil dinucleotide he came to the conclusion that the two mononucleotide groups are united through their carbohydrate groups, the formula representing a substituted disaccharide. Again, he found that when nucleic acid is heated with ammonia, adenine-uracil dinucleotide is obtained, evidently by hydrolytic rupture of its central nucleotide linkage. On the other hand, when nucleic acid is heated with a mineral acid "its central nucleotide linkage is not disturbed, but the two terminal nucleotide linkages are broken and uracil-cytosine dinucleotide is formed." Further observation showed him that uracil-cytosine dinucleotide produced both uracil and cytosine, but neither guanine nor adenine; it likewise formed both pyrimidine nucleosides, but neither of the two purine nucleosides and yielded no easily split phosphoric acid.

The presence of ferments or enzymes in the glandular organs of the body capable of producing hydrolysis, oxidation and deaminization—especially purine ferments—has been recognized for some years, and nuclein fermentation was understood to be carried on by a number of distinct physiological agencies, the individual enzymes being given names indicative of their action, such as nuclease, adenase, guanase, xanthine-oxidase and uricase. On this subject Jones and his associates have done much work, showing the distribution of the various enzymes in organs and tissues, character of the autolytic changes produced, and the conditions under which the changes occur. Thus, nuclease acting upon the nucleic acid of the thymus and yeast, as Levene has shown, can be broken down eventually into its four mononucleotides, the first step doubtless being the formation of dinucleotides. The mononucleotides may then undergo further change by the action of another enzyme (nucleotidase) losing phosphoric acid and forming the four corresponding nucleosides. The latter may then break down under the influence of a nucleosidase, into their component carbohydrate and base. Further, as Jones has found, the amino-purine derivatives can undergo deaminization through the action of guanase, adenase, guanosine-deaminase, adenosine-deaminase, etc., with formation of the corresponding oxy-purine derivatives.

Again, the nucleosides, through hydrolysis, may yield the free bases xanthine and hypoxanthine, under the influence of guanosine-hydrolase, adenosine-hydrolase, etc. These in turn can be oxidized to uric acid by xanthine-oxidase, thus giving a picture of possible autolytic changes in organs and tissues which help to explain many

metabolic phenomena. The distribution of these various ferments is of great interest. Most animal tissues, for example, can form hypoxanthine from adenosine either by *deaminization followed by hydrolysis, or by hydrolysis* followed by deaminization. In this connection it is interesting to note, as Jones states, that some tissues cannot form hypoxanthine from *free* adenine, although quite able to do so from *combined* adenine. Yeast alone was found to be exceptional: it could deaminize neither free nor combined adenine. Dog's liver, however, while it cannot form hypoxanthine from free adenine will form hypoxanthine quantitatively from yeast nucleic acid.

Numerous investigations have shown that adenase, guanase and xanthine-oxidase, while widely distributed through the organs of different animals, vary greatly in their localization with animal species, glands and age. Thus, in dog's liver, guanase is present, but not adenase, while pig's spleen contains adenase, but no guanase. Xanthine-oxidase, on the other hand, is not present in either of the above glands of those two animals, but is contained in ox liver. Finally, Jones came to the following conclusions regarding the distribution of purine ferments in ***human*** organs; uricase is not present in the liver, adenase is not present to any extent in any organ, guanase is contained in the kidney, liver and lung but not in the pancreas and spleen, while xanthine-oxidase is extremely active in the liver, but is not present in any other organ.

Turning now to another worker in this same field we find added light thrown on the nature of nucleic acid and its derivatives. Phoebus A. Levene had his early training in the universities of Russia, Switzerland and

Germany, coming to the United States in 1893. For ten years he served as chemist at the State Pathological Institute of New York, also working for a time at the Saranac Laboratory for the Study of Tuberculosis. Since 1907 he has been connected with the Rockefeller Institute for Medical Research. Endowed with great capacity for work, and possessed of rare skill and judgment as an experimenter, he has added much to our knowledge of the chemistry of proteins, nucleins, carbohydrates and enzymes.

Among some fifty of his papers dealing with nuclein compounds, the following may be cited as having special interest: *Darstellung und Analyse einiger Nucleinsäure,* eleven papers under this general title published in the *Zeitschrift für physiologische chemie,* during the years 1901-1908; *Über die Tritico-nucleinsäure,* with F. B. La Forge, 1910; *Über die Inosinsäure,* with W. A. Jacobs, 1908; *Über die Pentose in den Nucleinsäure,* and *Über Guanylsäure,* with W. A. Jacobs, 1909; *Über die Hefe-nucleinsäure,* with W. A. Jacobs, (4 papers) 1909-1911; *Über die Hefe-nucleinsäure,* with F. B. La Forge, 1912; *Über die Konstitution der Thymo-nucleinsäure,* with J. A. Mandel, 1908; all published in the *Berichte der Deutschen chemischen Gesellschaft; On Nucleases,* with F. Medigreceanu, 1911; *On the Structure of Thymus Nucleic Acid,* and *On Guanylic Acid,* with W. A. Jacobs, 1912; *The Structure of Yeast Nucleic Acid,* 1917; *On the Structure of Thymus Nucleic Acid and on Its Possible Bearing on the Structure of Plant Nucleic Acid,* 1921; all published in the *Journal of Biological Chemistry.*

The critical reader will at once notice in the above list of titles, several duplications, but under different dates,

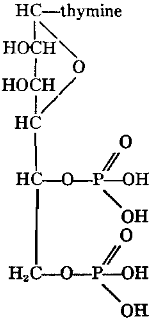
notably the "structure of thymus nucleic acid." This constitutes a good indication of the many complexities attending the study of the nucleins, suggesting as it does the necessity for repeated study before definite and final conclusions could be reached.

Lack of space prevents consideration of all the additions to our knowledge of nucleins made by Levene and his associates, but their studies bearing on the structure of the nucleic acids are of such importance that these must be discussed in some detail. It is to be remembered that there are certain fundamental differences between the nucleic acids of animal and vegetable origin, the former containing a hexose precursor and the latter a pentose. Further, plant nucleic acid contains a uracil group in place of the thymine group present in animal nucleic acid. In 1908, Levene, with Mandel, subjected nucleic acid from the thymus gland to partial hydrolysis, from which resulted a substance having the formula $C_{11}H_{17}N_2PO_{10}$. On submitting this substance to more vigorous hydrolysis they obtained thymine, levulinic acid and phosphoric acid. Later, in 1912, Levene and Jacobs obtained from animal nucleic acid by ferment action a substance having the composition $C_{11}H_{15}N_5O_6$, which on hydrolysis yielded guanine and hexose. The first of these two substances was thymine-nucleotide, while the second was guanine-nucleoside, *i.e.*, a nitrogenous ring compound united with hexose.

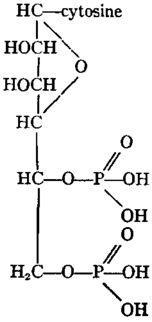
Four such nucleosides, combinations of hexose with cytosine, thymine, adenine and guanine respectively are possible products from the breaking down of nucleic acid. Further, through combination of these

nucleosides with phosphoric acid, four corresponding mononucleotides are conceivable as entering into the structure of the nucleic acid. Levene considered that the two pyrimidine nucleotides are united presumably by an ether linkage between the sugars. Further, each sugar of the thymine and cytosine nucleosides is conjugated with a secondary phosphoric acid, which in turn forms a bridge between the pyrimidine and the purine nucleosides.

This view was based upon the fact that after hydrolysis of nucleic acid with 2 per cent sulfuric acid for two hours, the purines were wholly removed and the sugar originally in union with them almost completely converted into levulinic acid. The resulting mixture was fractionated with phosphotungstic acid and from the portion not precipitated by this reagent crystalline brucine salts and barium salts of a hexo-cytidine diphosphoric acid and a hexo-thymidine diphosphoric acid were obtained, having the following structure:

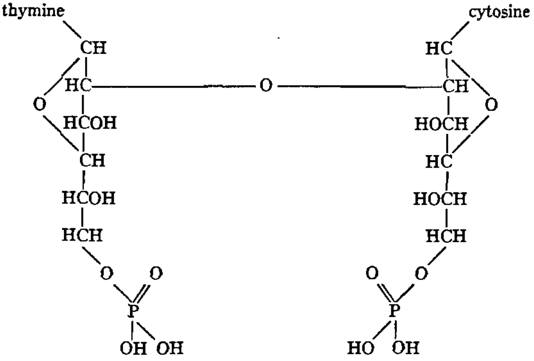


hexo-thymidine diphosphoric acid

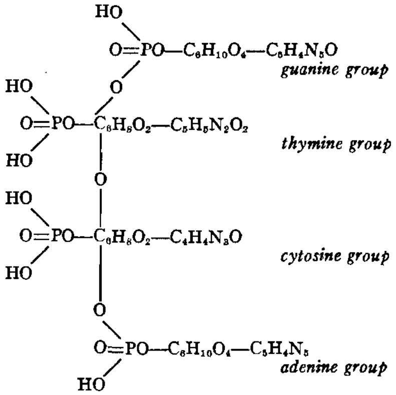


hexo-cytidine diphosphoric acid

From the phosphotungstic fraction, a dinucleotide was isolated, having the following structure:




From these and other observations, Levene arrived at the following structural formula as the probable constitution of animal nucleic acid, holding "that the nucleotides are united one to another in ester form, the phosphoric acid of one combining with the carbohydrates of the other."

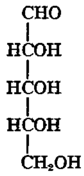



At this date no significance was attached to the configuration of the sugar, since its exact nature had not been determined.

Turning to plant nucleic acid, it is to be recalled that the chemical studies of this acid, ever since Kossel's original *work in 1893, had been made with the nuclein of* yeast, with one exception. In 1902, Thomas B. Osborne and his co-worker I. F. Harris, published in the *Zeitschrift für physiologische chemie,* the results of a very elaborate investigation of the wheat embryo, under the title *Die Nucleinsäure des Weizenembryos.* Thus, for the first time was established the presence in the embryo of wheat, and also probably in other seeds, of large amounts of a nucleic acid, to which they gave the name of *tritico-nucleic acid.* Of this new acid they made an exhaustive study, finding

among other facts that on hydrolysis it yielded adenine and guanine in equivalent amounts, also pentose and uracil, thus showing its resemblance at least to the nucleic acid from yeast. Later, cytosine was also found. On partial hydrolysis they obtained a substance rich in phosphorus, similar to the body obtained by Kossel from yeast nucleic acid, named by him plasmic acid. Osborne considered that like yeast nucleic acid, tritico-nucleic acid was built upon a polyphosphoric acid.

As a prelude to their work with yeast nucleic acid Levene and his associates gained important knowledge regarding inosinic acid and guanylic acid, which must be referred to. As is well known, inosinic acid by acid hydrolysis yields chemically equivalent amounts of hypoxanthine, phosphoric acid and pentose. Further, it had been found that the carnine of muscle extract was convertible by hydrolysis into hypoxanthine and a new body named inosine. Levene by neutral hydrolysis of inosinic acid obtained phosphoric acid and inosine, the same body that is formed from carnine. The carbohydrate of inosine, which Levene prepared from carnine and which he called carnose yielded an osazone identical with *d*-arabinosazone. Eventually he was able to show that carnose was *d*-ribose.



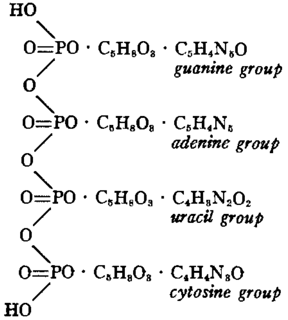
*d-ribose*

Working with the crystalline brucine salt of guanylic acid, Levene determined the composition of the free acid to be $C_{10}H_{14}N_5PO_8$, the relationship of guanylic acid to inosinic acid being the same as that of guanine to hypoxanthine.

| | | | |
|---|---|---|---|
| Guanylic acid | $C_{10}H_{14}N_5O_8P$ | Guanine | $C_5H_5N_5O$ |
| Inosinic acid | $C_{10}H_{13}N_4O_8P$ | Hypoxanthine | $C_5H_4N_4O$ |
| | NH | | NH |

On neutral hydrolysis of guanylic acid, a crystalline body was obtained having the formula $C_{10}H_{13}N_5O_5 . 2H_2O$, which when subjected to acid hydrolysis yielded guanine and *d*-ribose. Thus, it became clear that guanylic and inosinic acids are really mononucleotides composed of phosphoric acid and a purine base joined together by the pentose *d*-ribose. Following the same methods of procedure Levene and Jacobs subjected yeast nucleic acid to neutral hydrolysis at 175° C. under pressure and obtained the four nucleosides, adenosine, guanosine, cytidine and uridine; the two purine nucleosides, by simple hydrolysis being in turn converted into adenine and guanine respectively and *d*-ribose, while the two pyrimidine nucleosides yielded cytosine and uracil with *d*-ribose.

The structure of yeast nucleic acid is without much doubt indicated by the following formula, the work of Levene and his associates having done much to clarify and strengthen the generally accepted views on this subject.

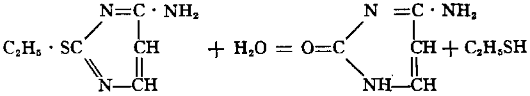



Finally, it may be added that Levene and La Forge working with tritico-nucleic acid were able to obtain from this acid by appropriate methods of hydrolysis adenosine, guanosine and cytidine, the same nucleosides that are present in yeast nucleic acid, together with *d*-ribose. Apparently, these two plant nucleic acids are essentially the same in structure, as originally suggested by Osborne.

In the Sheffield Laboratory of Organic Chemistry at Yale, Henry L. Wheeler and Treat B. Johnson in a series of synthetical studies established conclusively the structural make-up of all the known naturally occurring pyrimidines, *viz.*, uracil, thymine, cytosine and methyl-cytosine (from tubercle cell). The following papers may be cited: *Syntheses of Amino-oxy-pyrimidines having the Composition of Cytosine; 2-Amino-6-oxypyrimidine and 2-Oxy-6-aminopyrimidine,* H. L. Wheeler and T. B. Johnson, 1903; *On Some Condensation Products of the Pseudothioureas; Synthesis of Uracil, Thymine, and*

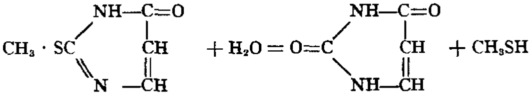
*Similar Compounds,* H. L. Wheeler and H. F. Merriam, 1903; *On Cytosine or 2-Oxy-6-aminopyrimidine from Tritico-nucleic Acid,* H. L. Wheeler and T. B. Johnson, 1903; published in the *American Chemical Journal. Researches on Pyrimidine Derivatives; 5-Methyl-cytosine,* H. L. Wheeler and T. B. Johnson, 1904; *Researches on Pyrimidines; 5-Ethyl-cytosine,* T. B. Johnson and G. A. Menge, 1906.

Starting with 2-ethyl-mercapto-6-oxy-pyrimidine, 2-ethyl-mercapto-6-chlor-pyrimidine was formed through the action of phosphorus pentachloride. By boiling this latter pyrimidine with alcoholic ammonia, the corresponding amide was obtained, which by saponification was transformed into 2-oxy-6-amino-pyrimidine or cytosine.



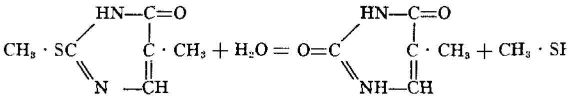
*cytosine*

Again, through condensation of formyl-acetic-ether with methyl-pseudothio-urea, methyl-mercapto-uracil was formed, which on boiling with a mineral acid yielded uracil.



*uracil*

By a similar method of treatment using formyl-propionic ether, they obtained 5-methyl-uracil or thymine.



*thymine*

Thus was confirmed the chemical structure of these pyrimidines originally assigned to them by Kossel.

In the Sheffield Laboratory of Organic Chemistry during the years 1904-1924 a large amount of work on pyrimidine compounds and their substitution products was accomplished by Professor Treat B. Johnson and his co-workers; studies in synthesis, some of which have physiological value. In all, sixty-eight papers appeared from the laboratory during this time, all bearing on the structure of pyrimidine derivatives. A few of these studies may be cited: *Some 5-Iodopyrimidine Derivatives; 5-Iodocytosine,* T. B. Johnson and C. O. Johns, 1906; *On Methods of Synthesizing Isobarbituric Acid, and 5-Oxycytosine,* T. B. Johnson and E. V. McCollum, 1906; *On the Formation of Purines from Urea-Pyrimidines,* T. B. Johnson and E. V. McCollum, 1906; *Synthesis of Thymine-4-carboxylic Acid,* T. B. Johnson, 1907; *Synthesis of 1-Methyl-5-hydroxyuracil,* T. B. Johnson and D. Breese Jones, 1909; *The Isomerism of 4-Phenyliso-cytosine,* T. B. Johnson and A. J. Hill, 1914; *Secondary-Pyrimidine-Nucleosides and Their Unique Behavior on Hydrolysis,* T. B. Johnson and S. E. Hadley, 1916; *The Behavior on Hydrolysis of the Simplest Nucleoside of Thymine,* T. B. Johnson and S. E. Hadley, 1917.

Of greater interest physiologically are the studies recently made by Treat B. Johnson and Elmer B. Brown

on the chemistry of the tubercle bacilli, especially of the nucleic acid; work which was rendered possible by aid from the Committee on Medical Research of the National Tuberculosis Association. In this connection the following papers by Johnson and Brown may be cited: *Nucleic Acid from Tuberculinic Acid,* 1922; *The Pyrimidines Contained in Tuberculinic Acid, the Nucleic Acid of Tubercle Bacilli,* 1922; *The Analysis of Tuberculinic Acid,* 1923; *The Sugar Contained in Tuberculinic Acid, the Nucleic Acid of Tubercle Bacilli,* 1923.

Various chemical studies have been made of tubercle bacilli, but largely, owing to the small quantities of material worked with, the results have not been very satisfactory. In this country, Levene in 1901 obtained from the crushed bacilli which had been freed from fat a phosphorus-containing substance which while not pure was undoubtedly a nucleic acid, since on hydrolysis it yielded not only phosphoric acid but also guanine and adenine. Johnson and Brown from their work obtained an acid from which they separated and identified the two pyrimidines, cytosine and thymine, and the purine adenine. Uracil was not found and guanine appeared only in the filtrates from the nucleic acid.

They came to the conclusion that tuberculinic acid, *i.e.,* a true tetronucleotide, is markedly unstable and that in the processes of purification the guanine nucleus is split off by hydrolysis, leaving a relatively stable trinucleotide. This latter nucleic acid yielded on hydrolysis adenine, cytosine, thymine, levulinic acid and formic acid, the two latter representing the carbohydrate group, which must be a hexose. Hence this nucleic acid from the tu-

bercle bacilli is related to the nucleic acids of animal origin; the absence of uracil and the presence of hexose instead of a pentose indicating clearly a structure different from that of known nucleic acids of the plant type.

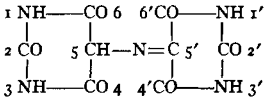
After removal of the nucleic acid from the tubercle bacilli, Johnson and Brown studied the character of the protein of the cells, noting particularly the distribution of the nitrogen, as revealed by the Van Slyke method of analysis, with the following results:

| | *Per Cent* |
|---|---|
| Amide nitrogen | 11.83 |
| Humin | 4.11 |
| Cystine | 1.26 |
| Arginine | 10.63 |
| Histidine | 11.48 |
| Lysine | 3.69 |
| Monamino nitrogen | 47.39 |
| Non-amino nitrogen | 9.34 |
| Tryptophane | present |
| | 99.73 |

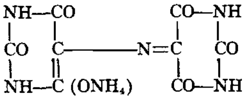
In a later study, *The Distribution of Nitrogen in the Protein Fraction of Tubercle Bacilli, after Removal of Tuberculinic Acid,* by Treat B. Johnson and Robert D. Coghill, 1925, the preceding results were confirmed, emphasizing the large percentage of basic amino-acids contained in the tubercle protein and showing a close relationship to the cell proteins contained in the cytoplasm of the leaves of such plants as spinach, alfalfa and ensilage corn. These same investigators also identified (1925) 5-methyl-cytosine as a product of hydrolysis when tuberculinic acid is digested with sulfuric acid.

Further studies of the tubercle bacillus are referred to in Chapter VIII.

The value of the "Murexide test" in the study of purine compounds has long been recognized, but the chemical structure of purpuric acid and consequently of murexide likewise has not been rightly understood. This was made quite clear by Professor Stieglitz [8] of the Kent Chemical Laboratory of the University of Chicago, working with Max Slimmer, who found that murexide dried at 100° C. is in reality the ammonium salt of an acid whose constitution is as follows:



Purpuric acid when free is not stable, but breaks down into uramil and alloxan. Murexide in its anhydrous form they found has the following constitution:



[8] *"The Constitution of Purpuric Acid and of Murexide,"* by Max Slimmer and Julius Stieglitz. *Am. Chem. J.* (1904).

# CHAPTER V

Establishment of a department of physiological chemistry at Columbia University—Connective tissue studies by William J. Gies—Chondroitin-sulfuric acid, Phoebus A. Levene—Studies on uric acid, Stanley R. Benedict—Thiasine or ergothioneine—Coagulation of the blood, William H. Howell—Origin of fibrinogen, experiments of Walter J. Meek—Work of G. H. Whipple, and E. W. Goodpasture—Crystallography of hemoglobins, Edward T. Reichert—Blood as a physico-chemical system, Lawrence J. Henderson—Studies of gas and electrolyte equilibria of blood by Donald D. Van Slyke—Work of Yandell Henderson on hemato-respiratory functions—Anglo-American Pike's Peak Expedition.

Glycoproteins, or glucoproteins as they are also termed, constitute a second group of conjugated proteins having considerable physiological and chemical importance. Widely distributed in the connective or supporting tissues such as tendons, cartilage and bone, present in the cornea, aorta and in mucous membranes as well as in their secretions, such as saliva and gastric mucus, the glycoproteins have special biological significance through their chemical relation to chitin, the chief component of the external and internal structures of the arthropoda. The first accurate knowledge of the chemical nature of these compound proteins, *i.e.*, of chondromucoid, tendomucoid, etc., came from C. T. Mörner of Sweden, 1889, who brought to light the fact that chondromucoid contains within its molecule a conjugated sulfuric acid, which eventually received the

name of chondroitin-sulfuric acid, the chondroitin containing a carbohydrate group.

Concerning these mucoids much knowledge has been gained by the work of several American investigators. Before considering this subject, however, it is necessary to refer to another matter connected with the development or expansion of physiological chemistry in America. In 1898 the authorities of Columbia University decided to establish a *Department of Physiological Chemistry* at the College of Physicians and Surgeons and I was requested to organize such a department for instruction and research. This I did, giving one day a week to the work for five years, then leaving the department in the hands of the men who had been trained to carry it on. This statement is worthy of record because it affords a good illustration of the growing interest in physiological chemistry which the medical schools of the country were beginning to manifest, for the College of Physicians and Surgeons of New York City as one of the large and influential medical schools of the country was taking a step which other schools were sure to follow.

In establishing this department at Columbia I naturally drew upon the New Haven laboratory for the personnel to care for the various needs of the new laboratory with its large body of students, the chief instructor being William J. Gies, who took his Ph.D. degree at Yale in physiological chemistry in 1897. In 1902 he was advanced to the rank of adjunct professor and in 1905 he was made professor of physiological chemistry. Two years later his title was changed to professor of biological chemistry.

For a number of years, 1901-1904, Gies and his asso-

ciates carried on a series of investigations on the chemistry of different forms of connective tissue, such as *Chemical Studies of Osseomucoid, with Determination of the Heat of Combustion of some Connective Tissue Glucoproteids,* with P. B. Hawk, 1901; *The Composition of Tendon Mucoid,* with W. D. Cutter, 1901; *Chemical Studies of Elastin, Mucoid and Other Proteins in Elastic Tissue, with Some Notes on Ligament Extractives,* with A. N. Richards, 1902; *On the Composition and Chemical Properties of Osseoalbumoid with a Comparative Study of the Albumoid of Cartilage,* with P. B. Hawk, 1902; *Do the Mucoids Combine with Other Proteids?,* with E. R. Posner, 1904; *A Preliminary Study of the Digestibility of Connective Tissue Mucoids in Pepsine-Hydrochloric Acid,* with E. R. Posner, 1904; all published in the *American Journal of Physiology.*

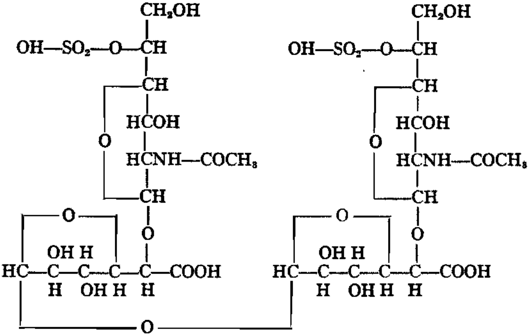
It is to be remembered that at this date not much was known regarding the details of composition of these tissues, consequently the results obtained had a certain value both for physiologists and chemists. Owing to the peculiar chemical make-up of the mucoids of these connective tissues, greater interest naturally centered about their chemical structure and relationship. On this subject Phoebus A. Levene of the Rockefeller Institute for Medical Research and his associates F. B. La Forge, G. M. Meyer, I. Matsuo, E. P. Clark and J. López-Suárez accomplished a large amount of work during the years 1913-1919, with results of the greatest significance.

Various European investigators, notably Schmiedeberg, have studied the nucleus of chondroitin-sulfuric acid with the general conclusion that it is made up of chondrosin

which when acetylated becomes chondroitin. Further, chondrosin was known to be composed of two substances related in some way to the carbohydrates; one view being that it resulted from the union of glucosamine and glucuronic acid, the two compounds being joined together by the attachment of the carbonyl group of the glucuronic acid to the amino group of the glucosamine. Many other views prevailed, however, all more or less divergent.

Levene with F. B. La Forge in four communications under the title, *On Chondroitin Sulphuric Acid,* published in the *Journal of Biological Chemistry,* 1913-1915, showed quite conclusively that the acid in chondroitin is glucuronic acid. Hydrolysis of chondrosin was accomplished most satisfactorily with sodium amalgam, the glucuronic acid being eventually identified by analysis of its phenylhydrazine and parabromophenylhydrazine compounds. Further, on oxidation of the products of hydrolysis of chondrosin with nitric acid, as well as by oxidation with bromine, saccharic acid was formed.

The other carbohydrate in chondrosin, Levene and La Forge found to be a new hexosamine, quite distinct from glucosamine but isomeric with it, to which they gave the name of chondrosamine. The formula which they ascribed to chondroitin-sulfuric acid recognizes the presence of four components in equimolecular proportion, *viz.,* acetic, sulfuric, glucuronic and chondrosaminic acids.



*chondroitin-sulfuric acid*

This formula, unlike the one assumed by Schmiedeberg, gives expression to the belief that in the disaccharide linkage a carbon atom of chondrosamine is joined with one of the hydroxyls of the glucuronic acid, in glucosidic linking. The structural formula as a whole is based upon the following experimental data: the carboxyl group of glucuronic acid as contained in chondrosin is free, oxidation of chondrosin with subsequent cleavage yields saccharic acid, the nitrogen of chondrosin is present as a primary amino group, the acetyl group is linked to the amino group of the sugar since all the nitrogen of chondrosin is in the form of free amino groups, while chondroitin-sulfuric acid does not contain such free groups. Finally, that two units are combined in a glucosidic linking between the glucuronic acids is predicated on the fact that while chondroitin-sulfuric acid does not have reducing action with Fehling's solution, chondrosin does.

In another series of experimental studies *On the Conjugated Sulphuric Acid from Tendomucoid,* 1914, Levene and La Forge found that the structure of the acids of this group was analogous to that of chondroitin-sulfuric acid. Freed from sulfuric acid a substance was formed, mucoitin, non-reducing and without free amino groups. By hydrolysis of this body mucosin resulted, a disaccharide composed of glucuronic acid and chitosamine or glucosamine. The configuration of the mucoitin-sulfuric acid molecule Levene considered to be the same as that of the corresponding chondroitin compound. Exceedingly interesting is the distribution of these two types of conjugate acids in the animal body. In an article, *Mucins and Mucoids,* with J. López-Suárez, 1918, Levene has given the details of preparation of the two conjugate acids from various organs and tissues, preparation and identification of the several cleavage products of each acid from the different sources, together with the analytical data obtained from the individual substances.

Thus, chondroitin-sulfuric acid was prepared from cartilage, tendons, aorta and sclera; mucoitin-sulfuric acid from funis mucin, vitreous humor, cornea, mucin of gastric mucosa, serum mucoid, ovomucoid and ovarian cysts. The wealth of material contained in the twenty-five distinct publications by Levene and his associates on this general subject offers a striking illustration of what can be accomplished by coöperative research in a properly endowed institution, when under competent leadership. The results of Levene's experimental work in this field have been brought together in a monograph of the Rockefeller Institute for Medical Research, No. 18, 1922, under

the title *Hexosamines, Their Derivatives, and Mucins and Mucoids*. Finally, it should be added that the monograph in question contains a large amount of work bearing on the configuration of 2-aminohexoses, synthesis of *d*-2-aminohexonic acids, conversion of hexosaminic acids into their epimers, optical rotation, etc.

Passing to another field of work, the relations of uric acid to metabolism, it is necessary to recall that uric acid is the most highly oxidized member of the purine group, that the purine portion of nucleic acids is the main source of uric acid in mammals, the many enzymes referred to in the preceding chapter being the active agents in the transformations that occur. The chemical structure of nucleic acids was solved by Levene, as has been pointed out, while the action of enzymes in the breaking down of nucleic acids was made clear by Walter Jones and his associates. Many other American workers have contributed to our knowledge of both the exogenous and endogenous formation of uric acid. Thus, Lafayette B. Mendel and John F. Lyman in the Sheffield Laboratory of Physiological Chemistry, 1910, reported that after the ingestion of free purine bases by man large proportions were excreted as uric acid; hypoxanthine 60 per cent, xanthine 50 per cent, adenine about 35 per cent, and guanine about 25 per cent.

Otto Folin's well-known work on the composition of normal urines, 1905, previously referred to, showed that the lowest possible level of uric acid excretion in man was attained on a diet very low in nitrogen but providing calories sufficient to protect the tissues of the body. Increasing the protein intake under these conditions does

mined each day, the total amount of uric acid eliminated during this period amounting to over 100 grams. As Benedict states, "not 10 per cent of this quantity of uric acid could have come from the pre-formed purines of the animal's tissues." Apparently, "this experiment is the first which definitely shows that an adult mammal can synthesize the purine nucleus." Likewise important was the observation that uric acid introduced under the skin of the Dalmatian is excreted quantitatively as such in the urine, while the output of allantoin is largely increased. Obviously, as Benedict suggests, it is "probable that uric acid and allantoin are inter-related in metabolism in other ways than have been heretofore assumed."

Some of these observations have been corroborated by H. G. Wells, of the University of Chicago, 1918, who in addition studied the action of various tissues of the Dalmatian dog on uric acid. Thus, he found that the liver possessed the power of destroying uric acid *in vitro*, which would indicate that the presence of uric acid in the urine of this breed of dog is not due to the absence of uricase in its tissues. The kidney, on the other hand, did not show *any uricolytic activity. Wells also found that while the* liver of the animal deaminized both adenine and guanine, neither the liver nor spleen could convert xanthine into uric acid.

Another interesting feature of Benedict's work has to do with uric acid in the blood, *On the Uric Acid in Ox and Chicken Blood,* 1915. While uric acid has been known to be present in the blood under pathological conditions ever since the work of Garrod eighty years ago, lack of suitable methods has long stood in the way of convenient and accu-

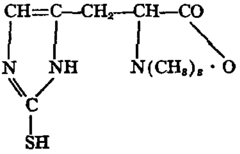
rate study of the possible presence of uric acid in human blood. In fact, until recent years, it was generally considered doubtful if the normal blood of man ever contained uric acid. To Otto Folin, of the laboratory of biological chemistry of the Harvard Medical School, the physiological chemist is indebted for many exact methods suitable for both scientific research and for clinical purposes. This is especially true of his method for the detection and estimation of uric acid in all kinds of blood.

Using the Folin method, Benedict has made some very important observations regarding uric acid in ox blood and chicken's blood, notably that in ox blood the larger portion of the uric acid present is combined in the corpuscles, while in chicken blood the uric acid is free and is contained almost entirely in the serum. To quote from Benedict, "In fresh ox blood, for instance . . . we find about one-half of one milligram of uric acid in 100 grams of blood. If, however, the blood filtrate after removal of protein is boiled with hydrochloric acid and then the uric acid determined, it is found that the quantity present is more than ten times that originally obtained. The same figure is ultimately reached if the whole blood is simply allowed to stand, thus indicating that an enzyme is present in blood which can split the uric acid combination." Finally, it is an interesting fact that the ox, which has little or no uric acid in the urine, has blood which contains 50 per cent more uric acid than is present in chicken blood, in spite of the fact that in the chicken uric acid is a conspicuous end-product of metabolism.

Especially interesting is the recent discovery by Benedict of *A New Sulphur-containing Compound* (*Thiasine*)

*in the Blood,* 1925, worked out in collaboration with Eleanor B. Newton and Jeannette Allen Behre. A provisional formula of $C_{12}H_{20}N_4O_8S$ was assigned to the compound, which was crystalline, contained no α-amino acid nitrogen and was *dextro*-rotary. In pig's blood and human blood it was contained wholly in the corpuscles, and on the basis of the earlier analyses the amount in human blood was placed as high as 15 milligrams per 100 cc. In diabetic cases the amount rose to 20-27 milligrams per 100 cc. of blood. By improved methods of determination, Behre and Benedict, in 1929, found that the average content of the compound in human blood was about 7.5 milligrams per 100 cc. with extreme variations between 4.2 and 15 milligrams per 100 cc. of blood

As to the chemical nature of thiasine, Benedict, Dakin and Newton, reported in the *Journal of Biological Chemistry,* 1927, that this compound is identical with the base ergothioneine isolated by Tanret from ergot in 1909, ergothioneine being the betaine of thiolhistidine with the following structure:




Treatment of thiasine by the methods used in elucidating the structure of ergothioneine gave results identical with those obtained from the latter.

The authors suggest that in view of the wide-spread

distribution among mammals of ergothioneine it would be better to drop the prefix "ergo" and use the term "thioneine" for this substance present in the blood, especially as this latter term "would serve to indicate the sulfur content of the compound, as well as the fact that the sulfur is present in the SH form as in cysteine."

Blood, which is frequently referred to as the circulating tissue of the body, is especially characterized by its ability under certain conditions of changing from a liquid to a jelly-like solid, or clot. Probably no physiological phenomenon has been more widely investigated than the coagulation of the blood, yet in spite of the extensive studies made no wholly adequate explanation of the various steps of the process has been offered. There are obviously many factors involved in the formation of blood-fibrin, as has been made quite clear by the early work of Schmidt and the later studies of Hammarsten and Wooldridge. Thus, consideration must be given to fibrinogen, a pre-existing substance in blood plasma; to thrombin which does not pre-exist in blood but appears only when clotting occurs and which in some unknown manner converts fibrinogen into fibrin; to prothrombin, the antecedent of thrombin, termed by some writers proferment or thrombogen, originating, it may be, in the blood plates; to antithrombin or some kindred substance and calcium salts, all of which are considered as playing some part in the process or processes of coagulation.

Among American workers in this field, William H. Howell, professor of physiology at The Johns Hopkins University, dean of the medical faculty of that University, 1899-1911, and since 1917 assistant director and later

director of the School of Hygiene of Johns Hopkins, stands out as one of the most prominent. His experimental work in physiology has been both broad and intensive but our interest is centered on his studies of problems connected with the coagulation of blood.

The following papers bearing on this subject have appeared from the physiological laboratory of The Johns Hopkins University: *The Proteids of the Blood with Especial Reference to the Existence of Non-coagulable Proteid,* by W. H. Howell, 1906; *The Coagulation of Blood,* by L. J. Rettger, 1909; *The Preparation and Properties of Thrombin, together with Observations on Antithrombin and Prothrombin,* by W. H. Howell, 1910; *The Intravenous Injection of Thrombin,* by Daniel Davis, 1911; *The Rôle of Antithrombin and Thromboplastin (Thromboplastic Substance) in the Coagulation of Blood,* by W. H. Howell, 1911; *The Presence of Prothrombin and Thromboplastin in the Blood Platelets,* by Stanhope Bayne-Jones, 1912; *The Nature and Action of the Thromboplastic (Zymoplastic) Substance of the Tissues,* by W. H. Howell, 1912; *Note on the Effect of Temperature upon the Action of Thrombin and Antithrombin,* by W. H. Howell, 1914; all published in the *American Journal of Physiology.*

From this large amount of material, it is possible here to note only a few of the more important findings. Pure thrombin prepared by Howell's method reacted like a simple protein, giving positive reactions with the biuret, Millon's and tryptophane tests but containing no sulfur or phosphorus. Solutions of thrombin to which some sodium chloride had been added could be heated to boiling

without losing completely their coagulating action on fibrinogen. Apparently, thrombin does not act upon fibrinogen after the manner of an enzyme; with increase of thrombin increased amounts of fibrin were formed, but in decreasing proportion.

Further, the amount of fibrin produced by a submaximal amount of thrombin was not modified by the length of time the thrombin was permitted to act. Noteworthy was the fact that one part of thrombin could convert at least 215 times its weight of fibrinogen into fibrin. As Howell states, it would appear probable that pure thrombin introduced directly into the circulation of an animal would cause a marked effect, but the results of such experiments were wholly negative, thus showing quite clearly that the body possesses some means of safeguarding itself against even large doses of thrombin.

As is well known, when peptone is injected into the circulation, blood plasma loses the power of clotting and the addition of even large amounts of thrombin to such plasma fails to bring about clotting. In other words, there is contained in peptone plasma something which holds the thrombin in check and prevents its ordinary action on fibrinogen, *i.e.*, an antisubstance or antithrombin. Howell found that this substance was susceptible to heat of 75°-80°C. Thus, if peptone plasma was heated to 60°C. for ten minutes the action of thrombin on fibrinogen was still prevented, but when the plasma was heated to the higher temperatures the power of antagonizing the action of thrombin was completely destroyed. Experiments by various investigators have indicated that not only in peptone plasma but also in the plasma of bird's blood and in

normal mammalian plasma a substance is present which prevents the action of thrombin on fibrinogen.

The fact that circulating blood does not clot is due not alone to the presence of an antithrombin but because the thrombin exists there in an inactive form, *i.e.*, as prothrombin. Two factors have been supposed to be involved in the formation of an active thrombin; one is calcium, the other an unknown organic substance, sometimes termed an activator or kinase, which converts the prothrombin into thrombin. The results of Howell's work seem to indicate, however, that the blood platelets and tissue extracts which are assumed to furnish this kinase, in reality contribute "a thromboplastic substance or thromboplastin which neutralizes the antithrombin, and thus permits the calcium to activate the prothrombin and start the process of clotting."

The hypothesis formulated by Howell, on the basis of his numerous experiments, recognizes three necessary fibrin factors, *viz.*, fibrinogen, prothrombin and calcium. "These substances are prevented from reacting, and the normal fluidity of the blood is maintained by the fact that *antithrombin is also present, and this substance prevents* the calcium from activating the prothrombin to thrombin. In shed blood the restraining effect of antithrombin is neutralized by the action of a substance (thromboplastin) furnished by the tissue elements. In the mammalia the thromboplastin is derived, in the first place, from the elements of the blood itself (blood platelets). In the lower vertebrates the supply of this material, in normal clotting, comes from the external tissues."

As to the chemical nature of the thromboplastic or

zymoplastic substance of the tissues, Howell has made many experiments confirming in part the results obtained by other investigators and furnishing additional data of importance. His results led him to the conclusion "that the thromboplastic substance is essentially the same in all tissues and acts in all cases in the same way." It is apparently an ether-soluble phosphatid, akin to kephalin, easily extracted from brain tissue, thymus and other dried tissues; it is likewise soluble in water, and the aqueous extract can be boiled without destroying its thromboplastic action. On long standing, however, the aqueous solution gradually loses its activity.

Apparently, the active thromboplastic substance, as it exists in an aqueous or saline extract of the thymus gland, for instance, is in combination with protein material, some form of tissue protein with a low temperature of heat coagulation. To this fact is due the observation of other workers that heating an aqueous extract of the tissues results in a destruction of the thromboplastic substance. In reality, Howell has found, such treatment leads to a coagulation of the protein matter of the extract, the thromboplastin being carried down with the heat coagulum with consequent loss of activity on the part of the filtered extract. The thromboplastin, however, is not destroyed by the heat, but is to be found in the coagulum in active form.

The origin of fibrinogen, the mother substance of fibrin, has been the subject of discussion and experiment ever since the early work of Claude Bernard in 1848. Among the many Americans who have studied this problem reference may be made to the work of Walter J. Meek, *Rela-*

*tion of the Liver to the Fibrinogen Content of the Blood,* from the physiological laboratory of the University of Wisconsin, 1912, and to the papers of G. H. Whipple, *Fibrinogen, an Investigation Concerning its Origin and Destruction in the Body,* and E. W. Goodpasture, *The Association of Liver and Intestine in Rapid Regeneration of Fibrinogen,* both from the Hunterian Laboratory of Experimental Pathology, Johns Hopkins Medical School, 1914, all published in the *American Journal of Physiology.*

That there is a rapid regeneration of fibrinogen, as when blood has been removed from the body by successive bleedings, there is abundant evidence, but just what agencies are responsible for renewal of the fibrinogen has not been clearly determined, although the liver has been generally credited with this power. In addition, the intestines have been found by some investigators apparently active in this direction, while bone marrow and the leucocytes have likewise been thought to be fibrinogen formers. Meek's experiments with dogs showed that after partial defibrination, fibrinogen may be regenerated to the extent of even 100 per cent in three hours; after the establishment of an Eck fistula and ligation of the portal vein, fibrinogen may still be formed after partial defibrination, but less rapidly; while with an Eck fistula and ligation of both portal vein and hepatic artery, fibrinogen was no longer reformed after partial defibrination, and the amount still in the blood quickly disappeared.

In Whipple's studies, observations were made upon the fibrinogen of the blood both in man and in the dog. He found that the fibrin antecedent varied normally in dog's plasma between 0.2 and 0.85 per cent, while in man the

normal limits were 0.3 to 0.6 per cent. The fibrinogen content was not influenced apparently by either starvation or feeding. As Whipple found that with injuries to the liver and in diseases of that organ, both acute and chronic, the fibrinogen of the blood dropped to a very low level, he inferred that the liver was probably not only quite active in the formation of fibrinogen but also that it "is the most important factor in maintaining a constant fibrinogen balance."

With the Eck fistula, together with ligation of the hepatic artery, constituting thereby "liver removal" he failed to observe any appreciable drop in the amount of fibrinogen in a period of five hours; with the "head-thorax circulation," by ligation of the aorta, vena cava, etc., there was a decided diminution of fibrinogen. The results in the "liver removal" experiments may indicate, Whipple believed, a reserve of fibrinogen or the production of fibrinogen by some other organ (*e.g.*, intestines) as there is no reason to suppose any sparing of fibrinogen more than in the "head-thorax circulation." Further, the "liver removal" experiment "when combined with defibrination shows that the body, excluding antithrombin and the liver, has powerful means at hand to neutralize large amounts of thrombin introduced into the circulation."

In Goodpasture's experiments, the plan followed was to study the rate of fibrinogen regeneration in pups completely defibrinated by perfusion, using for this purpose defibrinated blood from normal animals, this process being continued until samples of the animal's blood showed no detectable fibrinogen. Space does not permit any

lengthy presentation of his results and the conclusions drawn therefrom, but the following statements may be made. Pups treated as described showed a rapid reproduction of fibrinogen, a firm clot being formed in the blood. within thirty minutes after complete defibrination. Further, while ligation of the hepatic artery and spleen pedicle led to a slight reduction in fibrinogen regeneration, ligation of the intestine produced a marked delay in the reproduction of fibrinogen after complete defibrination. Again, after complete extirpation of the intestine in adult dogs, there was a return to the normal content of fibrinogen in eight hours. These and other facts led Goodpasture to the conclusion that "normal fibrinogen production is a result of the combined activity of the liver and the intestine; the intestine is not essential to fibrinogen regeneration, but is an important contributing factor in its rapid formation."

Another piece of work on the blood of a totally different character from the preceding emanated from the S. Weir Mitchell Laboratory of Physiology of the University of Pennsylvania, and had to do especially with the crystallography of hemoglobins. The research was commenced by Dr. Reichert in 1902 and the results were published by the Carnegie Institution of Washington in 1909, under the general title of *The Differentiation and Specificity of Corresponding Proteins and Other Vital Substances in Relation to Biological Classification and Organic Evolution; the Crystallography of Hemoglobins,* by Edward Tyson Reichert, professor of physiology, and Amos Peaslee Brown, professor of mineralogy and geology in the University of Pennsylvania.

Some idea of the magnitude of the investigation may be conveyed by the statement that there are one hundred plates each containing six photomicrographs of hemoglobin crystals in the report, these crystals coming from the blood of every conceivable species of animal. Thus, of the marsupials nine specimens were examined, including the opossum, Australian cat, the rat kangaroo and the Tasmanian devil; of the rodents eighteen species; twenty species of the ungulates, including the horse, mule, hippopotamus, antelopes, buffalo, blue sheep of Thibet, etc.; ten species of the Canidae, including dog, coyote, fox, wolf, jackal; nine species of the cats, including the lion, Bengal tiger, jaguar, puma, lynx, etc.; other Carnivora such as sea-lion, walrus, otter, polar bear; various species of Primates such as the ring-tail lemur; six species of baboons and man; various species of fishes, batrachiams and reptiles. Further, the specimens came from all over the world, with duplicates of a given species from different localities, so as to note the effect, if any, of distribution and environment on the hemoglobin of the blood.

In the preparation of the crystals of hemoglobin a uniform method of procedure was followed, consisting of the addition of oxalate to the blood in order to prevent coagulation, laking with ether to set the hemoglobin free, centrifugalizing the solution to free it from any extraneous matters, and then crystallizing on slides under covers sealed with Canada balsam. The crystals obtained were mostly oxyhemoglobin, but other forms were also observed in many cases, *e.g.*, metoxyhemoglobin, reduced hemoglobin, methemoglobin and occasionally carbon-monoxide hemoglobin. Especially noteworthy was the fact that in

many species the "fresh blood would first crystallize in one form of oxyhemoglobin; that later a second crop of crystals would appear having a totally different habit and even crystal system, or, in other words, different constitution; and that sometimes this would be succeeded by a third crop having a still different form." Thus, in the baboons three distinct crops of oxyhemoglobin crystals were observed, "tabular or lath-shaped orthorhombic crystals, short prismatic to tabular monoclinic crystals, and tabular orthorhombic crystals."

In the horse and mule the oxyhemoglobin crystallized in orthorhombic prisms and also in monoclinic tabular crystals; the CO-hemoglobin was likewise dimorphous and crystallized in the same respective systems. As the authors state, "not only is it possible for several different kinds of oxyhemoglobin, metoxyhemoglobin, reduced hemoglobin, CO-hemoglobin and methemoglobin to occur in the same species, but that these five different substances may be distinguished from each other by crystallographic characters as well as by spectroscopic examination."

The fact that hemoglobin does not crystallize in the corpuscles of the blood Reichert and Brown assumed to be due to the osmotic properties of the stroma, "which keep the solvent at too low a percentage in the corpuscle to allow of crystallization, while it does not crystallize from the plasma in the living animal because the solution is too dilute or the temperature too high for that dilution." Quite probably the hemoglobin and the stroma are united in some kind of combination. The research taken as a whole represents a large amount of painstaking work, the value of which to the physiologist and the chemist can

hardly be overestimated. The work was made possible by grants from the Carnegie Institution of Washington.

The study of blood as a physico-chemical system and as a tissue has been the subject of numerous investigations in this country during the past twenty years, notably by Lawrence J. Henderson, professor of biological chemistry in Harvard University, and by Donald D. Van Slyke, research biological chemist of the Rockefeller Institute for Medical Research. As Henderson has expressed it, blood is a relatively simple system, essentially free from all forms of metabolic activity, enzymatic processes, etc., and likewise free from all movement—excluding the amæboid-like movements of the leucocytes—except that which is imparted from without and hence is well suited to quantitative experimental study.

In the earlier years there was lack of the advantages which have come from the developments of the theory of solutions, the law of mass action, the ionic theory by which so many hitherto unexplainable facts have been made clear, together with the introduction of physico-chemical habits of thought and physico-chemical methods of experimentation; but Van Slyke and Henderson have both carried on with great success elaborate series of studies using the most modern methods and conceptions, which have done much to advance our understanding of such matters as the nature of the homogeneous and heterogeneous equilibrium of the blood, the respiratory cycle, acid-base equilibrium, effect of oxygenation on the acid-base properties of hemoglobin, effect of carbon dioxide on the distribution of acid and base, etc.

The following experimental studies by Henderson may

be cited as indicating in some degree the character of the work he and his associates have accomplished: *Equilibrium in Solution of Phosphates*, 1906; *Concerning the Neutrality of Protoplasm*, with O. F. Black, 1907; *A Study of the Equilibrium between Carbonic Acid, Sodium Bicarbonate, Mono-sodium Phosphate and Di-sodium Phosphate at Body Temperature*, with O. F. Black, 1908; *A Theory of Neutrality Regulation in the Animal Organism*, 1908; *On the Union of the Proteins of Serum with Alkali*, 1908; *On the Neutrality Equilibrium in Blood and Protoplasm*, 1909; *A Critical Study of the Process of Acid Excretion*, 1911; all published in the *American Journal of Physiology.*

As is clearly understood, the proper working of physiological processes is dependent upon the accurate adjustment and preservation of physico-chemical conditions within the organism. As Henderson stated in 1911 "three such conditions are now known to be nicely adjusted and maintained, *viz.*, temperature, molecular concentration and neutrality." The metabolic processes of the body are yielding continuously excretory products with acid reaction, notably carbonic acid, sulfuric acid and phosphoric acid, which immediately, according to their several avidities, combine more or less freely with the basic components of the blood and protoplasm.

The early experiments of Henderson with Black showed that the phosphates of protoplasm are especially concerned in the neutralization of acid produced by or introduced into the cell and that they are able to accomplish this result with very little change in hydrogen ionization, even though considerable amounts of acid have to be cared

for. Thus, it was found that in the presence of both free and combined carbonic acid at 20°C., mono- and di-sodium phosphates can exist only in molecular proportions varying between 1:9 and 5:5 approximately. All such solutions were precisely neutral, of hydrogen ionization $1 \times 10^{-7}$ nearly. Hence, as Henderson stated, "protoplasm is accordingly extraordinarily safeguarded by the presence of phosphates and carbonates in considerable amount from variation in hydrogen or hydroxyl ionization. The occurrence of alkalinity is absolutely prevented by the presence of free carbonic acid, and the system can neutralize relatively enormous quantities of acid without losing its precise neutrality." Again, it was found that the hydrogen ionization of blood serum corresponds to that of solutions of mixtures of $Na_2HPO_4 + NaH_2PO_4$ in which the ratio of the two constituents varies between 6:4 and 1:0 approximately, *i.e.*, variations presumably quite harmless.

In Henderson's *Theory of Neutrality Regulation*, 1908, it was shown theoretically and experimentally that both protoplasm and blood increase in alkalinity with rise of temperature, the hydrogen-ion concentration holding nearly constant, "so that the alkalinity (concentration of hydroxyl ions) of blood in the body is probably about three times as great as it has been believed to be. Moreover, there is probably an increase of about one-fourth in alkalinity when the normal body temperature rises to that of high fever.

These relationships are due to the fact that the water constant $(C = (\overset{+}{H}) \times (\overline{OH}))$ increases much more rapidly with the temperature than do the ionization constants of carbonic acid, and other weak acids which can

take part in the equilibrium in the body." Further, it was shown that the physiological mechanism for the preservation of neutrality, "or more precisely of a condition in which approximately $(\overset{+}{H}) = 0.4 \times 10^{-7}$ N and $(\overline{OH}) = 7.2 \times 10^{-7}$ N at 38° in the aqueous solutions of the body, possesses a remarkable and unsuspected degree of efficiency." This efficiency is due to the "avidity of carbonic acid and mono-sodium phosphate as acids and on their high diffusibility, on heterogeneous equilibria selectively adjusted, and on the mechanism for the excretion of carbonic acid and for the regulation of osmotic pressure."

Passing on to a later period we find a long series of studies by Henderson and his associates under the general title of *Blood as a Physicochemical System*, published in the *Journal of Biological Chemistry,* covering a large amount of experimental work carried on in the laboratory of biological chemistry of the Harvard Medical School and in the medical laboratories of the Massachusetts General Hospital. In one of the early papers of this series, 1923, emphasis was laid upon the use of a two dimensional nomographic chart upon which could be represented the concentration of free and combined oxygen, of free and combined carbonic acid of the serum, of serum chloride and of the hydrogen ion.

Henderson found that the relationship between the six variables was such that for a given blood, when values are assigned to any two, the values of the other four are determined and the condition of equilibrium unequivocally defined. Hence, the nomogram "illustrates the known facts

regarding the acid-base equilibrium of blood, the oxygen dissociation, the carbon dioxide dissociation, the distribution of chlorides and the influence of oxygen upon the affinity of hemoglobin for base." It would appear that all six of these variables are involved in a single physicochemical equilibrium, and that in the respiratory change of the blood all six are important factors.

In a still later investigation, 1923, with A. V. Bock, H. Field, Jr., and J. L. Stoddard, Henderson presented a more elaborate and detailed study of all the changes known to occur in normal human blood during the respiratory cycle, in which a general or synthetic description in the form of a nomogram of the physicochemical factors recognized as involved in the complex equilibrium of the blood were charted. There was also given a detailed or analytic description, with the aid of many contour line charts, of those relations between the several factors which conveniently lend themselves to separate consideration; likewise a study of the implications of the diffusion theory concerning the respiratory exchanges in lung and tissue capillaries. Thus was established in part at least the thesis "that the components of the system of which consideration is necessary and sufficient for the proper treatment of the respiratory changes are $H_2O$, $O_2$, $CO_2$, serum base, cell base, serum protein, cell protein and Cl and H ions."

Between 1923 and 1927 many other investigations under the general title *Blood as a Physicochemical System*, several of them from the medical laboratories of the Massachusetts General Hospital, came from Henderson and his associates. Of these, two may be noted, *viz., The Oxygen and Carbon Dioxide Dissociation Curves of Human*

*Blood,* 1923; and *The Composition and Respiratory Exchanges of Normal Human Blood During Work,* 1927. Incidentally it may be stated that in the first of these two investigations it was found that both corpuscles and plasma transport carbon dioxide, the former carrying 40 per cent of the total and the latter 60 per cent.

Finally, it should be added that the foregoing statements regarding Lawrence J. Henderson's experimental work on blood give a very imperfect and incomplete picture of the great breadth and scope of his undertaking, of the extreme difficulty, and intricacy of the experimental procedures upon which his conclusions are based, and the skill displayed in overcoming these obstacles to progress. It should also be stated that the constant coöperation which existed between Henderson and Donald D. Van Slyke contributed much to the successful outcome of the work of each. In 1928 Henderson gave the Silliman Lectures at Yale for that year, taking for his subject *Blood, a Study in General Physiology,* the book which followed giving a comprehensive review of his own work and that of his collaborators.

An interesting study of the blood of certain invertebrates merits a brief notice, *viz., The Transport of Oxygen and Carbon Dioxide by Some Bloods Containing Hemocyanin,* by Alfred C. Redfield, Thomas Coolidge and Archer L. Hurd, 1926, from the Harvard physiological laboratory and Woods Hole. Does hemocyanin—a copper-protein compound—function as an oxygen carrier in the blood in the same manner as the hemoglobin of vertebrate blood? What is the general nature of the union between the hemocyanin and the gases of the blood? Do the hemo-

cyanins of various groups of invertebrates show specific differences? These are among the questions the above investigation aimed to answer. The conclusions reached as a result of the study of the general conditions of equilibrium between oxygen, carbon dioxide, etc., in the blood of four species of invertebrates indicated that "the properties of the hemocyanin of each of these species are distinctive, but that all of these proteins function in the transport of oxygen and carbon dioxide according to the same physicochemical principles as obtained in the case of hemoglobin."

Passing now to the work of Donald D. Van Slyke and his associates, conducted mainly in the laboratories of the Hospital of the Rockefeller Institute for Medical Research, we find recorded under the general title *Studies of Gas and Electrolyte Equilibria in the Blood,* a number of very important physico-chemical investigations, from which data of great physiological value were obtained. Among these several studies, all published in the *Journal of Biological Chemistry,* the following may be cited: *The Alkali-binding and Buffer Values of Oxyhemoglobin and Reduced Hemoglobin,* by Donald D. Van Slyke, A Baird Hastings, Michael Heidelberger and James M. Neill, 1922; *The Effect of Oxygenation and Reduction on the Bicarbonate Content and Buffer Value of Blood,* by Donald D. Van Slyke, A. Baird Hastings and James M. Neill, 1922; *Studies of Gas and Electrolyte Equilibria in the Blood,*[1] by Donald D. Van Slyke, Hsien Wu and Frank-

[1] From the laboratory of physiological chemistry and the department of medicine, Peking Union Medical College, Peking, China, and the Hospital of the Rockefeller Institute for Medical Research.

lin C. McLean, 1923; *The Acid Properties of Reduced and Oxygenated Hemoglobin,* by A. Baird Hastings, Donald D. Van Slyke, James M. Neill, Michael Heidelberger and C. R. Harington, 1924; *The Distribution of Hydrogen, Chloride and Bicarbonate Ions in Oxygenated and Reduced Hemoglobin,* by Donald D. Van Slyke, A. Baird Hastings, Cecil D. Murray and Julius Sendroy, Jr., 1925.[2]

From the many results of these experimental studies and the concepts drawn therefrom it is possible here to refer only to a few. Regarding the laws governing the distribution of electrolytes and water between blood cells and serum, these were subjected to experimental verification. On the assumption "that the laws holding in dilute solutions for the relationships between the reaction and the amounts of base bound by weak and strong acids, the distribution of diffusible and non-diffusible electrolytes on two sides of a membrane, and the proportionality between the ratio

$$\frac{\text{molecules} + \text{ions of solute}}{\text{molecules of water}}$$

and the osmotic pressure are also valid for blood, mathematical expressions have been derived which predict the distribution of electrolytes and water between cells and serum, and the manner in which the distribution is affected by changes in pH and oxygen content." The effects of varying $CO_2$ tension were likewise investigated with results approximating those predicted.

Their many data also made clear that the base bound by the cell proteins of oxygenated horse blood over the

[2] For the related work of John P. Peters, see Chapter VIII.

physiological pH range can be expressed approximately in milli-equivalents by the equation

$$[B\,P]_c = 3.6\ [H\,b]_c\ (pH_c - 6.6)$$

when H b expressed millinols of hemoglobin in terms of oxygen capacity.

Further, the base bound by the proteins of the serum is indicated, over the physiological range of reaction, by the equation $B\ P_s = 0.068\ P_s\ (pH_s - 4.80)$ where $P_s$ expresses grams of serum protein. It was also found that the osmolar concentrations in the blood cells and in the serum are equal when calculated as

$$\frac{\text{molecules} + \text{ions of solute}}{\text{water}}$$

the electrolytes being assumed to be equally dissociated in the cells and serum.

Regarding the alkali-binding power of hemoglobin, it was found that the alkali (Na) bound at pH 7.4 per gram molecule of recrystallized oxyhemoglobin from horse blood was $2.15 \pm 0.10$ equivalents, while that per gram molecule of reduced hemoglobin was $1.47 \pm 0.08$. "At pH 7.4 the change of one mol of reduced hemoglobin to oxyhemoglobin enables the hemoglobin to combine with $0.68 \pm 0.10$ equivalent of additional alkali." As to the variation in base bound by oxyhemoglobin at varying pH, Van Slyke found this was indicated by the molecular buffer value of $\beta_O = 2.64$. "For each 0.1 pH increase, each molecule of oxyhemoglobin takes up 0.264 additional equivalents of base. For reduced hemoglobin the molecular buffer value $\beta_R$ is 2.45. The molecular buffer values, $\beta_O =$

2.64 and $\beta_R = 2.45$ indicate that at pH 7.2 to 7.5 at least five monovalent acid groups in the molecule share in the alkali combined with hemoglobin."

The oxygenated blood of the horse was found to have an average buffer value at pH 7.2 to 7.5 $\frac{d\,\beta\,(m\,M)}{d\,pH} = 25.3$, while in the reduced condition it was 24.4. In the oxygenated blood, it was calculated that the hemoglobin was responsible for an average of 76.0 per cent of the total buffer value, while bicarbonate was responsible for 6.9 per cent. In reduced hemoglobin the figures given were 73.3 and 9.0 respectively. Another fact to be noted is that the isoelectric point, $I_R$, of reduced horse hemoglobin, that is the point at which equal amounts of base and acid are bound, is at pH 6.81 ± 0.02.

*Finally, the following statement may be recorded as* expressing one of the important general conclusions drawn by Van Slyke from certain phases of his experimental work. "Hemoglobin combines within itself the ability to combine and release almost an entire molecule of oxygen at atmospheric and tissue tensions; ability so to change its base-binding power with oxygenation and reduction that the base released is equivalent to a large part of the $CO_2$ normally exchanged for oxygen; and a high buffer value at physiological pH range. In possessing these three properties combined, balanced and active within physiological gas tension and reactive ranges, hemoglobin shows unique adaptation to its function as carrier of carbon dioxide and oxygen."

The work which Van Slyke and his associates have carried forward so successfully in a field calling for accurate

physiological and chemical knowledge, combined with broad experience, affords another striking illustration of the advance which physiological chemistry is making in America.

Another worker in this same field but from a somewhat different angle is Yandell Henderson, professor of applied physiology in Yale University. Henderson took the degree of Ph.D. in physiological chemistry at the Sheffield Scientific School of Yale University in 1898, after which he served for a year as assistant in physiological chemistry, becoming then instructor in physiology, assistant professor of physiology in the Yale Medical School 1903-1911, professor of physiology 1911-1921, when his title was changed to professor of applied physiology. He also had the advantage of experimental work in physiological chemistry, with Kossel at Marburg and with Cremer at Munich.

While Henderson's investigations have been varied in character he has contributed much to knowledge of the hemato-respiratory functions, the general trend of his studies in this field being indicated by the following brief selection from his publications, contained in the *Journal of Biological Chemistry: Respiratory Regulation of the* $CO_2$ *Capacity of the Blood, High Levels of* $CO_2$ *and Alkali,* with Howard W. Haggard, 1917; *The Fallacy of Asphyxial Acidosis, How Oxygen Deficiency Lowers the Blood Alkali,* with Howard W. Haggard, 1920; *The Reversible Alterations of* $H_2CO_3 : NaHCO_3$ *Equilibrium in Blood and Plasma under Variations in* $CO_2$ *Tension and Their Mechanism,* with Howard W. Haggard, 1920; *Respiration and Blood Alkali During Carbon Monoxide As-*

*phyxia*, with Howard W. Haggard, 1921; *The Buffering of the Tissues as Indicated by the $CO_2$ Capacity of the Body*, with R. J. Brockelhurst, 1927.

It is not possible here to discuss all the results of these and kindred studies of the hemato-respiratory functions, but it may be noted in general that Henderson was led to the belief, "that the blood is a physiological fluid regulated by a living organism and not merely a physico-chemical system." Regarding the relation of oxygen to blood alkali he lays stress upon the importance of the oxygen pressure in the lungs. "The amount of alkali in use in the blood of a healthy individual is fundamentally regulated and determined by the pressure of oxygen in his lungs at the altitude at which he lives. In other words, the type of blood that he has in his vessels at any one time is a function of the mean barometer at the place he is then living." Again, "the barometric pressure to which one becomes acclimatized, through the tension of oxygen, is the fundamental factor controlling the volume of air breathed per unit mass of $CO_2$ eliminated, the alveolar $CO_2$ tension, and the amount of alkali called into use in the blood."

Further, Henderson's observations led to the conclusion that in the asphyxia produced by carbon monoxide, alkalosis results and not acidosis; the lowering of blood alkali is due to the acapnial not the acidotic process. Acidosis in the sense of low pH, Henderson believes, is simply depression of breathing, and that its more fundamental causes lie somewhere in the regulation of respiration. Any reasonable degree of low blood alkali can easily be compensated by a quite feasible increase of

breathing. "For all ordinary degrees of 'acidosis' in the sense of low blood alkali, the immediate, sufficient and sole cause of the low pH is the (relative) depression of the respiratory center and of the volume of breathing."

Further, in the *American Journal of Physiology* is a long list of papers containing the results of Henderson's work on another aspect of respiration, from which the following may be noted, under the general title of *Acapnia and Shock: Carbon Dioxide as a Factor in the Regulation of the Heart Beat,* 1908; *Fatal Apnoea after Excessive Respiration,* 1910; *Acapnia as a Factor in the Dangers of Anaesthesia,* with Marvin McRae Scarbrough, 1910; *Failure of the Circulation in Acapnia,* 1910; *Failure of Respiration After Intense Pain,* 1910; *The Circulation and its Measurement,* with Howard W. Haggard, 1925; *The Maximum of Human Power and its Fuel,* with Howard W. Haggard, 1925. While most of these latter studies are apparently more physiological than chemical in character, yet in all of them the experimental work involved was largely chemical in nature, having to do with the analysis of the blood gases, determination of the $CO_2$ content of the blood, etc., and aiming to throw light upon the chemical regulation of respiration.

The experiments of Henderson and Haggard point quite clearly to the fact that inhalation of $CO_2$ in slightly more than the alveolar concentration is very effective in restoring a low blood alkali to normal after anaesthesia. The latter condition is not only attended with a decided decrease in the alkali reserve, but the hydrogen-ion concentration of the blood is at the same time increased, accompanied by depressed respiration. One of the practical out-

comes of Henderson's work has been the use of carbon dioxide as a means of resuscitation from carbon monoxide asphyxia, from ether or alcohol intoxication and from respiratory failure due to other causes; the inhalation of carbon dioxide tending to restore the blood alkali and the hydrogen-ion concentration to normal values.

Especially interesting and important were the studies carried on by Henderson and Edward C. Schneider in coöperation with J. S. Haldane and G. Gordon Douglas, of Oxford University, in the Anglo-American Pike's Peak Expedition, 1911, "planned by members of Oxford and Yale Universities, with the main object of making a thorough study of physiological adaptation to low atmospheric pressures." The expenses of the expedition were met in part by contributions by the Council of the Royal Society from the Donation Fund and by Yale University from the Loomis Medical Research Fund. Edward C. Schneider, the American member of the expedition with Henderson, was trained in physiological chemistry in the Sheffield Scientific School at Yale, receiving the Ph.D. degree in 1901. At the date of these studies he was professor of biology at Colorado College, and since 1919 professor of biology at Wesleyan University.

The laboratory which was installed near the summit of the peak was 14,093 feet above sea-level and was provided with suitable apparatus for determining the oxygen pressure of the arterial blood, percentages of oxygen and carbon dioxide, total respiratory exchange, pulse rates, systolic and diastolic, changes in the blood, such as the hemoglobin content, etc. The observations[3] made on this

[3] The full report of this expedition was published in the *Philosophical Transactions of the Royal Society of London*, B, 203: 185-318 (1913).

expedition—all made on man—showed that in the process of adaptation to high altitudes there are three definite changes, *viz.*, a rise in the arterial oxygen pressure; a fall in alveolar $CO_2$ pressure and corresponding rise in alveolar oxygen pressure; an increase in the percentage and total amount of hemoglobin in the blood.

The changes in $CO_2$ and O alveolar pressure were apparently due to diminished alkalinity of the blood, brought about by "some adaptive alteration in the regulation of blood alkalinity." As stated in the official report, "What actually occurs is that diminished *fixed* alkalinity is compensated for by diminished concentration of $CO_2$, with the result, as Barcroft first showed, that the dissociation curve of oxyhemoglobin is sensibly unaltered; and presumably also the total reaction or hydrogen-ion concentration is also almost unaltered, while the advantage of a raised alveolar oxygen pressure is secured."

# CHAPTER VI

Studies at the University of Pennsylvania, John Marshall, Philip B. Hawk, Alonzo E. Taylor and collaborators, D. Wright Wilson, Florian A. Cajori, Walter G. Karr—New York University and Bellevue Hospital Medical College, Holmes C. Jackson and John A. Mandel—Post Graduate Medical School, Victor C. Myers, Morris S. Fine—Teachers College, Columbia University, Mary Swartz Rose, Walter H. Eddy—The work of Henry G. Sherman, Columbia University—Christian A. Herter and the Herter Laboratory—Studies of Henry D. Dakin on oxidation and reduction—Washington University, Philip A. Shaffer, antiketogenesis —University of Michigan, Victor C. Vaughan and Frederick G. Novy, Howard B. Lewis—Carl P. Sherwin at Fordham University—*Henry C. Eckstein, University of Michigan*—University of Minnesota, Ross A. Gortner, Jesse F. McClendon—University of Rochester, Walter R. Bloor, and his work on lipoids—University of Wisconsin, Harold C. Bradley.

In 1904 the new medical buildings of the University of Pennsylvania were completed, and commodious well equipped laboratories were provided for the department of physiological chemistry. John Marshall taught medical chemistry at Pennsylvania, with one period of interruption, from 1878 to 1922, at first as demonstrator in "practical chemistry" under Theodore G. Wormley, who at that date was professor of chemistry and toxicology in the medical school of the university. From 1897 to 1922, Marshall himself held the chair of chemistry and toxicology in the medical department. He was one of those

Americans who early sought the advantages of European training, studying chemistry (1879-1882) with the renowned Wöhler at Göttingen, with Hüfner at Tübingen and at the University of Christiania. Both by training and ability he was well fitted to accomplish much in the field of physiological chemistry, but administrative duties covering many years as dean of the medical school and of the faculty of veterinary medicine, combined with other duties as head of his department of study, prevented the realization of his hopes. With his influence and broad knowledge, he was, however, a decided help in fostering interest in the subject at the University of Pennsylvania.

In 1903, Philip B. Hawk was called in as demonstrator in physiological chemistry, serving for four years, at the end of which time he became professor of physiological chemistry at the University of Illinois, 1907-1912; in the latter year he returned to Philadelphia as professor of physiological chemistry and toxicology at the Jefferson Medical College, 1912-1922. Philip B. Hawk was a graduate of Wesleyan University, 1898, the following two years being spent as assistant in chemistry to Wilbur O. Atwater. He then came to the Sheffield Scientific School where he pursued the study of physiological chemistry for two years, taking the M.S. degree in 1902. Consequently, Hawk, when he began his work at the University of Pennsylvania, was well fitted to help forward the development of physiological chemistry at that important center. His research work while there, and later on, testifies to his extreme diligence. Especially important was his text book, *Practical Physiological Chemistry,* the first edition of which appeared while he was at the University of

Pennsylvania, a book which has been widely used and which has been broadly helpful.

The next important advance in the development of physiological chemistry at the University of Pennsylvania came with the appointment of Alonzo E. Taylor as Rush professor of physiological chemistry in 1910. Taylor had his early training at Cornell College and De Pauw University, later at the University of Berlin, and in Sweden, under Arrhenius. In 1894 he took the M.D. degree at the University of Pennsylvania. From 1899 to 1910 he was professor of pathology and physiological chemistry at the University of California, and from the Hearst Laboratory of Pathology came the results of many of his investigations, such as *Chemical Studies in Cytolysis*, 1908; *On the Composition and Derivation of Protamin*, 1908; *On the Synthesis of Protamin through Fermentation*, 1908; all published in the *Journal of Biological Chemistry*.

While at Pennsylvania Taylor was extremely active, having the advantage of the coöperation of several younger men who were beginning to make names for themselves in the field of physiological chemistry; notably A. I. Ringer, who was instructor 1911-1913 and assistant professor of physiological chemistry 1913-1915; William C. Rose, instructor 1911-1913, and Howard B. Lewis, instructor 1913-1915. The two latter had taken the Ph.D. degree in physiological chemistry at the Sheffield Scientific School and each had served there as assistant in the laboratory for two years.

Of the scientific work which came from the Laboratory of Physiological Chemistry up to 1916, the following studies may be noted: *The Utilization of Ammonia in the Pro-*

*tein Metabolism,* by A. E. Taylor and A. I. Ringer, 1913; *Studies in the Purin Metabolism,* by A. E. Taylor and William C. Rose, 1913; *On Uricolysis,* by A. E. Taylor and W. H. Adolph, 1914; *The Influence of Protein Intake upon the Formation of Uric Acid,* by A. E. Taylor and William C. Rose, 1914; *A Study of the Protein Metabolism under Conditions of Repeated Hemorrhage,* by A. E. Taylor and H. B. Lewis, 1915; *The Chemistry of Glucogenesis; The Rôle of Pyruvic Acid,* by A. I. Ringer, with the aid of E. M. Frankel and L. Jonas, 1913; *The Formation of Glucose from Valerianic and Heptylic Acids,* by A. I. Ringer and L. Jonas, 1913; *The Formation of Glucose from Dioxyacetone in the Diabetic Organism,* by A. I. Ringer and E. M. Frankel, 1913; *Theory of Diabetes, with Consideration of the Probable Mechanism of Antiketogenesis and the Cause of Acidosis,* by A. I. Ringer, 1914; all published in the *Journal of Biological Chemistry.*

During the war Taylor's active connection with the university ceased and as a member of the War Trade Board he was engaged in various important matters, especially those connected with the United States Food Administration. In 1921 he became a director of the Food Research Institute at Stanford University. Finally, it should be mentioned that Taylor's book, *Digestion and Metabolism; the Physiological and Pathological Chemistry of Nutrition,* 1912, gives a scholarly presentation of existing knowledge at that date covering the field in question.

As successor to Taylor, D. Wright Wilson was appointed Benjamin Rush professor of physiological chem-

istry in 1922. He had been trained in the Sheffield Scientific School at Yale, receiving the Ph.D. degree in physiological chemistry in 1914. From 1914 to 1922 he was at The Johns Hopkins University as assistant, then associate, and during 1917-1922 associate professor of physiological chemistry. The following papers may be cited as illustrative of the character of his research work: *On the Determination of Free Amino Nitrogen in Proteins,* 1923; *Studies in Pyrimidine Metabolism,* 1923; *Changes in the Composition of the Urine after Muscular Exercise,* with several collaborators, 1925; *The Excretion of Lactic Acid in the Urine after Muscular Exercise,* with S. H. Liljestrand, 1925; published in the *Journal of Biological Chemistry.*

As co-workers in the laboratory, James C. Andrews was appointed instructor in 1922, becoming assistant professor of physiological chemistry in 1926, Florian A. Cajori being likewise appointed assistant professor. Andrews received the Ph.D. degree at Columbia University in 1918, while Cajori pursued the study of physiological chemistry in the Sheffield Scientific School at Yale, taking the Ph.D. degree in 1920. Both were active in research, Cajori having studied *The Effect of Changes in the Circulation on Carbohydrate Utilization,* 1925; *A Comparison of the Rate of Glycolysis in Different Bloods with Special Reference to Diabetic Blood,* 1924; *The Chemical Composition of Synovial Fluid in Cases of Joint Effusion,* 1927, and Andrews *The Optical Activity of Cystine,* 1925; *The Oxidation of Cystine,* 1925; *The Optical Activity of Cysteine,* 1926.

As further evidence of the growth of physiological

chemistry at the University of Pennsylvania, Walter G. Karr was appointed assistant professor of biochemistry in the graduate school of medicine in 1922, becoming later associate professor. Trained in physiological chemistry in the Sheffield Scientific School at Yale, taking the degree of Ph.D. in 1920, he became chief biochemist of the Philadelphia General Hospital in 1921, and has been occupied especially in clinical biochemistry. In 1924, George E. Simpson was also appointed assistant professor of physiological chemistry at Pennsylvania. A graduate of the University of Illinois, 1913, he was granted the Ph.D. degree in physiological chemistry at Yale in 1920, after which he became assistant professor of physiological chemistry at McGill University, 1920-1924. He has been especially active in the study of the water balance of the body.

At New York, in addition to the developments in physiological chemistry at the Cornell University Medical College and the Medical School of Columbia University previously referred to, other signs of progress were manifest marking the transition from an older to a newer and more modern viewpoint. Thus, at New York University and Bellevue Hospital Medical College, John A. Mandel had been appointed professor of chemistry and physiological chemistry in 1898, while in 1901 Holmes C. Jackson became instructor and 1902-1905 assistant professor of physiological chemistry with Mandel. Later in 1909 Jackson was assigned to the chair of physiology in the same institution, which position he held until his death in 1927. Mandel had his training in chemistry at New York University and at the University of Berlin, while Jackson had studied in the Sheffield Scientific School at Yale, tak-

ing the Ph.D. degree in physiological chemistry in 1899. Mandel had translated Hammarsten's *Text Book of Physiological Chemistry* from the author's German edition, thus rendering available for American students a book that was very helpful to those interested in the science. Jackson had prepared a manual of *Physiological Chemistry* for use in his laboratory courses, and both were active in research.

At the Post Graduate Medical School and Hospital, Victor C. Myers was appointed professor of pathological chemistry, 1912-1922, professor of biochemistry, 1922-1924, while Morris S. Fine was instructor, 1911-1914, and adjunct professor of pathological chemistry, 1914-1917. Both had been trained in the Sheffield Laboratory of Physiological Chemistry, taking the Ph.D. degree in 1909 and 1911 respectively. During their period of service in the Post Graduate Medical School they devoted themselves especially to an experimental study of the metabolism of creatine and creatinine. Ten or more papers came from their laboratory dealing with this general subject, all published in the *Journal of Biological Chemistry,* among which may be mentioned, *The Creatine Content of Muscle under Normal Conditions; Its Relation to Urinary Creatinine,* 1913; *The Influence of the Administration of Creatine and Creatinine on the Creatine Content of Muscle,* 1913; *The Relationship between Creatine and Creatinine in Autolyzing Tissue,* 1915; *The Creatine Content of the Muscles of Rats Fed on Isolated Proteins,* 1915; Myers at this date is professor of biochemistry at Western Reserve University.

At Teachers College, Columbia University, Mary

Swartz Rose began her work in the science of nutrition, first as instructor in 1909, advancing by successive steps until in 1923 she became the professor of nutrition. A graduate of Teachers College (B.S., 1906) she studied physiological chemistry with special reference to nutrition at the Sheffield Scientific School, being granted the Ph.D. degree in 1909. Through her work at Columbia and through her numerous writings, *Laboratory Handbook for Dietetics*, 1912, *Feeding the Family*, 1916, *Everyday Foods in War Time*, 1918, *The Foundations of Nutrition*, 1927, she has accomplished much in the field of dietetics and home economics, a form of applied physiological chemistry which promises much in aiding the people of the country to understand rightfully the basic principles which underlie human nutrition.

At about this same date Walter H. Eddy was appointed professor of physiological chemistry at Teachers College. A graduate of Columbia, Ph.D., 1909, research chemist at New York Hospital, he became assistant professor of physiological chemistry at Teachers College in 1919 and was advanced to the professorship in 1921. His research work has been mainly in connection with vitamines and will be referred to in a later chapter.

Another force in the field of nutrition at Columbia University was and is Henry C. Sherman, who since 1924 has been the Mitchill professor of chemistry. A graduate of that university, Ph.D., 1897, he has been connected with Columbia ever since that date, having the title professor of food chemistry, 1911-1924. In his earlier years he was connected with the nutrition investigations of the United States Department of Agriculture and for

a time was associated with Wilbur O. Atwater. He has carried on many investigations dealing with the composition of foods and other problems connected with human nutrition. The following titles may be noted: *The Balance of Acid-forming and Base-forming Elements in Foods,* with A. O. Gettler, 1912; *Monthly Metabolism of Nitrogen, Phosphorus and Calcium in Healthy Women,* with L. H. Gillett and H. M. Pope, 1918; *Phosphorus Requirement of Maintenance in Man,* 1919; *Protein Requirement of Maintenance in Man,* 1919; *Calcium Requirement of Maintenance in Man,* 1920; *The Calcium Content of the Body in Relation to Age, Growth and Food,* with F. L. MacLeod, 1925; *The Phosphorus Content of the Body in Relation to Age, Growth and Food,* with E. J. Quinn, 1926; all published in the *Journal of Biological Chemistry.* Especially valuable are his two books, *Chemistry of Food and Nutrition,* 1911, rewritten 1918 and 1926; and *Food Products,* 1914, 1924.

As a result of one hundred and nine experiments upon the protein needs of the body, Sherman found an indicated requirement of 0.635 gram of protein per kilogram of body weight, which for an "average man" of 70 kilograms would mean 44.4 grams per day. In ninety-five experiments upon phosphorus requirement the mean result was 0.88 gram per day, while in ninety-seven experiments upon calcium requirement the mean result was 0.45 gram per day.

Another step in the advance of physiological chemistry in New York, one of somewhat unusual character, was the establishment by Dr. Christian A. Herter of a private laboratory for experimental work, from which came

with astonishing regularity for many years the results of a great variety of highly important investigations carried on by him and his associates, especially H. D. Dakin. The work accomplished by Herter, directly and indirectly, and the influence he exerted in various directions, call for more than a passing notice. A graduate in medicine of Columbia University, trained in chemical pathology and the pathology of nutrition, professor of pathological chemistry at the University and Bellevue Hospital Medical College, 1897, professor of pharmacology and therapeutics at Columbia University, 1903 to the time of his death in 1910, he was keenly alive to the many ways in which chemistry might prove of service to scientific medicine.

To facilitate his own researches and to provide a place for other workers imbued with the same scientific spirit he himself possessed, a large and well equipped laboratory was arranged on the upper floors of his large house on Madison Avenue, where he drew about him a group of collaborators proficient in analytical methods and who understood the intricacies of organic chemistry, especially in the field of synthesis. He likewise established and financed *The Journal of Biological Chemistry* in 1905, as previously noted, and in this journal through many years appeared the legend *From the Laboratory of Dr. C. A. Herter, New York,* followed in later years, after his death, by the simpler form *From the Herter Laboratory,* at the head of many papers coming from this source. To most physiological chemists of the eastern seaboard 819 Madison Avenue was a well known locality.

Herter was especially interested in the chemical proc-

esses of the gastro-intestinal tract induced by micro-organisms and among his many studies the following may be cited: *On Alterations in the Composition of the Blood Resulting from Experimental Double Nephrectomy,* with A. J. Wakeman, 1899; *The Color Reactions of Napthaquinone Sodium-monosulphate and Some of Their Biological Applications,* 1905; *On Gas Production by Fecal Bacteria Grown on Sugar Bouillon,* with Herbert C. Ward, 1906; *A Method for the Quantitative Determination of Indol,* with M. Louise Foster, 1905; *On a Relation between Skatol and the Dimethyl-amidobenzaldehyde (Para) Reaction of the Urine,* 1905; *The Production of Methyl Mercaptan by Fecal Bacteria Grown on a Peptone Medium,* 1906; *On Bacterial Processes in the Intestinal Tract in Some Cases of Advanced Anaemia, with Special Reference to Infection with B. Aerogenes Capsulatus,* 1906; *On the Separation of Indol from Skatol and their Quantitative Determination,* with M. Louise Foster, 1906; *The Occurrence of Skatol in the Human Intestine,* 1907; *The Relation of Nitrifying Bacteria to the Urorosein Reaction of Nencki and Sieber,* 1908; *On Indolacetic Acid as the Chromogen of the "Urorosein" of the Urine,* 1908.

Herter was one of the "Referee Board of Consulting Scientific Experts" created by President Roosevelt, and in his laboratory one of the experimental studies entitled *The Influence of Sodium Benzoate on the Nutrition and Health of Man* was conducted, the other two studies on the same subject being conducted by John H. Long in Chicago, and by Russell H. Chittenden in New Haven, the report of the joint work being published in 1909 as

*Report No. 88 of the United States Department of Agriculture.*

Among other papers from the laboratory attesting the activities of various members of the Herter group in a wide range of studies, the following may be noted: *Acetonuria Following Chloroform and Ether Anaesthesia,* by Helen Baldwin, 1905; *The Formation of Glyoxylic Acid,* by H. D. Dakin, 1906; *The Glyoxylic-Acid Reaction for Tryptophan, Indol and Skatol,* by H. D. Dakin, 1906; *Changes in the Bile Occurring in Some Infectious Diseases,* by Helen Baldwin, 1908; *Estimations of Arginin, Lysin and Histidin in Products of Hydrolysis of Various Animal Tissues,* by Alfred J. Wakeman, 1908; *Bacillus Infantilis and its Relation to Infantilism,* by Arthur I. Kendall, 1909; *On the Decomposition of β-Oxybutyric Acid and Aceto-acetic Acid by Enzymes of the Liver,* by Alfred J. Wakeman and H. D. Dakin, 1909.

For years physiologists, in considering the problems of metabolism in the animal economy, were content with the general understanding that the foodstuffs, proteins, fats and carbohydrates were oxidized to certain simple end-products, *viz.*, carbon dioxide, water and urea. Gradually, there crept in added knowledge which made it quite clear that there were many intermediate steps of great physiological significance which needed explanation. The problems of intermediary metabolism thus began to attract the attention of both physiologists and chemists and as knowledge of chemical structure became clearer, the way was opened for unravelling many of the details of the simpler processes of oxidation and reduction, which take place in the animal body. The study of metabolism con-

sequently took on added importance, and the physiological chemist began to envisage a future when it would be possible to trace out in orderly manner the successive chemical reactions which make up the complicated processes of metabolism.

While oxidation and reduction are obviously the special agencies at work it is to be remembered that the substances which are continuously undergoing metabolism in the animal organism are quite resistant to oxygen under ordinary conditions. Even oxygen as present in oxyhemoglobin is without any noticeable oxidizing power and consequently it becomes apparent that in the animal body oxidation is not the simple process that might be inferred.

Among the many views advanced to explain the types of oxidation brought about by animal tissues, the suggested formation of unstable superoxides, acting somewhat like hydrogen peroxide, had much in its favor. As Dakin wrote in 1922, "It has been commonly assumed that the oxidases concerned with biochemical oxidations represent systems composed of a superoxide formed by the union of ordinary oxygen with an 'oxygenase' together with a catalyst (peroxidase) capable of acting upon it with production of active oxygen of high potential." What I wish to emphasize, however, is that the oxidations and reductions taking place in the animal body are of a peculiar order and that in the attempt to acquire all possible facts bearing on the processes by which these changes are brought about a large volume of work has been done by many investigators, among whom, in this country, H. D. Dakin stands out as one of the most productive.

Henry D. Dakin, educated in England, a student at Heidelberg, where he worked with Kossel, research chemist in the C. A. Herter laboratory, 1905-1920, and since the latter date in his private laboratory, Scarborough-on-Hudson, has been a most productive worker in the field of organic chemistry with special reference to biological applications. By his skill in experimentation and his scientific acumen he has been able to add much to knowledge of the complicated chemical reactions taking place in intermediary metabolism.

Among his many published articles dating from 1906 the following are selected, both for their particular significance and as representative of the character of his work: *The Oxidation of Amino-acids,* 1906; *Experiments Bearing upon the Mode of Oxidation of Simple Aliphatic Substances in the Animal Organism,* 1907; *Comparative Studies of the Mode of Oxidation of Phenyl Derivatives of Fatty Acids by the Animal Organism and by Hydrogen Peroxide,* 1908; *The Mode of Oxidation in the Animal Organism of Phenyl Derivatives of Fatty Acids,* 1909; *On the Decomposition of Acetoacetic Acid by the Enzymes of the Liver,* with A. J. Wakeman, 1910; *Experiments Relating to the Mode of Decomposition of Tyrosine and of Related Substances in the Animal Body,* 1910; *The Catabolism of Histidine,* with A. J. Wakeman, 1911; *The Formation of Amino and Hydroxy Acids from Glyoxals in the Animal Organism,* with H. W. Dudley, 1914; *An Enzyme Concerned with the Formation of Hydroxy Acids from Ketonic Aldehydes,* with H. W. Dudley, 1913; all published in the *Journal of Biological Chemistry.*

It is obviously impossible here to give any adequate

review of Dakin's work, but reference may be made to the results of several investigations bearing on types of oxidation taking place in the animal body. Thus, following the subcutaneous injection of an aqueous solution of the sodium salt of β-phenylpropionic acid, Dakin detected in the urine β-phenyl-β-oxypropionic acid, acetophenone with some benzoic acid. Similar injection of the sodium salt of β-phenyl-β-oxypropionic acid was followed by the presence in the urine of acetophenone, benzoic acid, together with some of the original acid unchanged. From these results Dakin drew the conclusion that β-phenylpropionic acid undergoes oxidation in the animal body—in part at least—as follows:

$$C_6H_5 \cdot CH_2 \cdot CH_2 \cdot COOH$$

*β-phenylpropionic acid*

$$\downarrow$$

$$C_6H_5 \cdot CHOH \cdot CH_2 \cdot COOH$$

*l-β-phenyl-β-oxypropionic acid*

$$\downarrow$$

$$C_6H_5 \cdot CO \cdot CH_2 \cdot COOH$$

*benzoylacetic acid*

$$\downarrow$$

$$C_6H_5 \cdot CO \cdot CH_2$$

*acetophenone*

$$\downarrow$$

$$C_6H_5COOH$$

*benzoic acid*

In studying the derivatives of phenylvaleric acid, Dakin found that the subcutaneous injection of the sodium salt of this acid gave rise to the presence in the urine of phenyl-β-oxypropionic acid, cinnamoyl glycocoll and acetophenone; these substances all being intermediary products in the catabolism of phenylpropionic acid, which by

further oxidation yield hippuric acid. In other words, when phenylvaleric acid is introduced subcutaneously, phenyl-$\beta$-oxyvaleric acid is the first or intermediate step in the process of catabolism, from which come the substances named above, the hippuric acid thus resulting by a very indirect process of oxidation. By such and similar experimental evidence it became apparent that the oxidation occurred in such fashion "that the five-carbon atom side-chain was converted primarily into a three-carbon atom side-chain and the latter again oxidized with a further loss of two carbon groups."

By repeated experiments with various related substances sufficient evidence was secured to warrant the view that acids of the type Ph·C·C·C·C·C·COOH undergo oxidation in the animal body in such manner that four carbon atoms are removed from the side chain in *two* pairs, in every case benzoic acid being the end product.

$$Ph\cdot C\cdot \vdots\, C\cdot C\cdot \vdots\, C\cdot COOH \rightarrow Ph\cdot C\cdot \vdots\, C\cdot COOH \rightarrow Ph\cdot COOH$$

This type of oxidation Dakin termed successive $\beta$-oxidation, and he considered it might well be a general biochemical reaction.

As an illustration of another type of reaction in which reduction results reference may be made to the experiments of Wakeman and Dakin with aceto-acetic acid. When the sodium salt of this acid was introduced intravenously into cats and dogs there was found in the urine levoratory $\beta$-oxybutyric acid, *i.e.*, a reduction takes place somewhere in the body, whereby the larger portion of the aceto-acetic acid is transformed into *l*-$\beta$-oxybutyric acid.

They also found that on digesting finely divided liver tissue outside the body with sodium aceto-acetate there was likewise a formation of *l*-β-oxybutyrate, thus indicating that the reaction is enzymatic in character. Since it is known that β-oxybutyric acid and substances giving rise to this acid may undergo oxidation in the liver with formation of aceto-acetic acid and acetone, it is clear that we have here a form of reversible reaction.

Wakeman and Dakin found that the reaction involved in the oxidation of β-oxybutyric acid was due to an oxidizing enzyme whose action was facilitated by the presence of free oxygen and of oxyhemoglobin or blood. Hence, as stated by Dakin, "it will thus be seen that the liver possesses a mechanism dependent upon the antagonistic action of two ferments by which the mutual interconversion of β-oxybutyric acid and aceto-acetic acid may be effected. The one ferment action is an oxidation dependent upon the presence of free oxygen or oxyhemoglobin, while the other ferment action must be of the nature of a reduction."

$$CH_3\cdot COHO\cdot CH_2\cdot COOH \underset{\textit{reductase}}{\overset{\textit{oxidase}}{\rightleftharpoons}} CH_3\cdot CO\cdot CH_2\cdot COOH$$

Undoubtedly many substances that take part in the synthetical processes going on in tissue cells are exceedingly labile and consequently are prone to change under slight provocation, a fact no doubt responsible for many metabolic phenomena. Glyoxal is a typical representative of unstable bodies of this type and Dakin thought it

would prove instructive to study the behavior of structurally related substances rendered partially stable by the incorporation of an aromatic group. He found that phenyl glyoxal given to rabbits is followed by the excretion of *l*-mandelic acid together with hippuric acid. The benzoic acid (hippuric acid) might, Dakin thought, originate either by direct oxidation of the phenyl glyoxal or by oxidation of the mandelic acid, or possibly by both methods.

$$\begin{array}{l} C_6H_5 \cdot CO \cdot CHO \rightarrow C_6H_5 \cdot CHOH \cdot COOH \rightarrow C_6H_5 \cdot COOH \\ \qquad \downarrow \\ C_6H_5 \cdot COOH \end{array}$$

That this is a general reaction is at least suggested by the fact that aqueous extracts of various tissues, such as the liver, pancreas, kidney, spleen, brain and muscle, all showed the presence of an enzyme able to transform phenyl glyoxal into mandelic acid, the reaction being readily inhibited by heat. As Dakin stated, "the fact that a ketonic aldehyde through enzyme action may unite with water to form an optically active hydroxy-acid appears to have some significance." As to the part ketonic aldehydes play in metabolism there is no definite information, but the fact that methyl glyoxal is readily formed from sugar by mild hydrolysis suggests that if this reaction is reversible this type of reaction might be a means for the formation of sugar from lactic acid and indirectly from amino-acids. In this connection it is suggestive to note that Dakin prepared from dog's liver an enzyme-containing solution which when added to pure methyl glyoxal converted it completely into lactic acid within ten minutes.

The work of Dakin has done much to make it clear that the various processes of oxidation and reduction which occur in the living organism are closely intertwined with other forms of reaction such as condensation and hydrolysis, all contributing to the complexity of intermediary metabolism. Finally, it should be added that Dakin has written a monograph entitled *Oxidations and Reductions in the Animal Body,* one of the Monographs on Biochemistry, edited by Plimmer and Hopkins, the second edition of which appeared in 1922, in which is contained a summation of the principal chemical reactions taking place in the body, "treated simply from the standpoint of the structure of the substances undergoing change."

In 1915, the new buildings of the School of Medicine of Washington University, St. Louis, were completed with improved facilities for the study of physiological chemistry. Under Philip A. Shaffer, professor of biochemistry since 1910, physiological chemistry has gained a conspicuous position in this university, much research work of varied character having been carried on in his laboratory. A pupil of Otto Folin of Harvard University, Ph.D. 1904, with experience gained by service in several scientific centers, Shaffer has through ability and diligence accomplished much. Various methods for the determination of organic substances in blood, urine and milk have come from his laboratory, but perhaps his most outstanding piece of work has been the study of antiketogenesis, published in 1921, in the *Journal of Biological Chemistry.*

The following papers may be referred to: *Antiketogenesis,* I. *An in Vitro Analogy;* II. *The Ketogenic-antiketo-*

*genic Balance in Man;* III. *Calculation of the Ketogenic Balance from the Respiratory Quotient;* IV. *The Ketogenic-antiketogenic Balance in Man and Its Significance in Diabetes; The Excretion of Acetone from the Lungs,* with A. P. Briggs.

In searching for a chemical explanation of antiketogenesis, Shaffer made the suggestive discovery that while hydrogen peroxide oxidizes aceto-acetic acid slowly in an alkaline solution, the addition of glucose to the mixture causes the aceto-acetic acid to disappear rapidly, the rate of disappearance increasing with increase in the amounts of glucose and alkali. In strongly alkaline solution at 38° C. with an excess of glucose and hydrogen peroxide the oxidation is complete in a few hours. Glucose was thus shown to possess in alkaline solution *in vitro* a ketolytic action in hastening the oxidation of aceto-acetic acid, which, as Shaffer stated, "appeared to be analogous to its antiketogenic action in the body. Glycerol and fructose were also found to be ketolvtic, while lactic acid had no such power.

These observations, fitting in as they do with the well-known fact that during fasting or with the simple omission of carbohydrate from the diet for some days, acetone appears in the breath while acetone, aceto-acetic acid and $\beta$-hydroxybutyric acid appear in the urine as in severe diabetes, led Shaffer to the view that the effect of carbohydrate in preventing or abolishing ketonemia in man probably is the result of a definite chemical reaction in the tissues. This might well be a reaction between some derivative of glucose and aceto-acetic acid, involving definite molecular quantities of each substance.

Starting with the hypothesis that antiketogenesis is due "to a chemical reaction in the body between definite and constant amounts of ketogenic and antiketogenic compounds analogous to the ketolytic reaction between aceto-acetic acid and glucose," Shaffer developed a trial method for calculating the molecular amounts of these two classes of compounds derivable from protein, fat and carbohydrate, assuming that each molecule of fat is convertible into 3 molecules of aceto-acetic acid and 0.5 molecule of glucose or its equivalent of antiketogenic derivative; that protein is convertible (a) into glucose or its equivalent to the extent of 3.6 grams for each gram of urine nitrogen and (b) into aceto-acetic acid for each molecule of leucine, phenylalanine and tyrosine, each gram of urine nitrogen being assumed to correspond to approximately 10 millimols of ketogenic substance; that carbohydrate exerts its antiketogenic action in the form of glucose or other hexose, 1 gram of which is equivalent to (1,000 ÷ 180 =) 5.56 millimols of antiketogenic substance.

Applying these calculations to subjects excreting small amounts of acetone bodies, Shaffer found that the hypothesis was essentially correct and that the minimum molecular ratio of ketogenic to antiketogenic substances for avoiding ketonuria in different human subjects was 1; a conclusion that was confirmed by calculation of data from a case of "total" diabetes with extreme acidosis, "the total hydroxybutyric acid excretion being approximately accounted for and in fair agreement with the calculated expectation." The conclusion drawn from the evidence collected was that "a molecular ratio of 1:1, which corresponds (according to the method of calculation) to a

respiratory quotient of 0.76 is the limit for the avoidance of the excretion of acetone bodies. With quotients higher than 0.76 the catabolism of the antiketogenic glucose (or its equivalent from protein and glycerol) is great enough to remove aceto-acetic acid as fast as it is formed, presumably by a 'ketolytic' reaction analogous to what occurs in the test tube."

Shaffer's calculations, made with a large number of published metabolism experiments, in which he gives the millimols of glucose and of fatty acids oxidized together in the mixture of metabolites are certainly impressive and give confidence in his general conclusions. Especially noteworthy is the fact that each molecule of glucose is capable of causing the complete oxidation of two molecules of fatty acid. It is now possible to calculate approximately at least the *minimum* amount of carbohydrate food needed to provide a theoretical ketogenic balance for any given subject; and this figure represents the absolute minimum of carbohydrate tolerance below which ketosis is *unavoidable*. The following formula is given by Shaffer as the most useful:

$$\frac{\left(\begin{array}{l}\text{Total calories of energy}\\ \text{exchange per 24 hours}\end{array}\right) - (100 \times \text{urine N})}{50} = \begin{array}{l}\text{Gram food CH}\\ \text{to provide ap-}\\ \text{proximate keto-}\\ \text{genic balance}\end{array}$$

In studying the excretion of acetone from the lungs, Shaffer found that the distribution coefficient for water and air in the vicinity of 37° C. and 750 mm. was about 334 for $\frac{\text{water}}{\text{air}}$. By the same methods the distribution coefficient of acetone for blood serum and air was ascer-

tained to be about 337. Again the concentration of acetone in urine was found to be essentially the same as that of whole blood and blood plasma, while the ratio of acetone in blood to that in alveolar air was about 333. The distribution of acetone between alveolar air and blood of human diabetics and of normal fasting subjects averaged 355.

At the University of Michigan physiological chemistry was welcomed early, Victor C. Vaughan having been appointed professor of physiological and pathological chemistry and associate professor of therapeutics and materia medica in 1883. From 1887 to 1909 he served as professor of hygiene and physiological chemistry and director of the hygienic laboratory. A graduate of the University of Michigan, Ph.D., 1876, M.D., 1878, lecturer in medical chemistry 1879-1880, Vaughan especially in the early years of his career was active in the development of physiological chemistry at the University of Michigan. As a student he had come under the teaching and influence of Albert B. Prescott, for many years director of the chemical laboratory of the University and consequently he was well trained in chemistry and especially well fitted to carry on the work which occupied his attention for a long period, *viz.*, the chemical study of leucomaines, ptomaines and toxins in general.

Closely associated with him was Frederick G. Novy, likewise a graduate of the University of Michigan, Sc.D., 1890, M.D., 1891, a student at Berlin, Prague and Paris, instructor in hygiene and physiological chemistry at Michigan 1887-1891, assistant professor 1891-1893, junior professor 1893-1902 and since the latter date pro-

fessor of bacteriology. In 1909, he succeeded Vaughan as director of the hygienic laboratory.

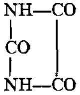
For many years the names of Vaughan and Novy were associated in various lines of work where knowledge of bacteriology and chemistry was needed, the results of their studies being embodied in a book, the first edition of which appeared in 1888, entitled *Ptomains, Leucomains, Toxins and Antitoxins, or the Chemical Factors in the Causation of Disease,* under their joint authorship. Especially noteworthy was the work of Vaughan on *tyrotoxicon* and on the toxins of typhoid fever. Again, Novy's book, *Laboratory Work in Physiological Chemistry,* second edition in 1898, did much to facilitate the study of the subject at Michigan and elsewhere.

Among the many workers in the hygienic laboratory at Michigan, mention should be made of Sybil May Wheeler who with the counsel and guidance of Vaughan carried on many experiments of chemico-physiological importance. The following may be cited: *The Chemistry of Sarcina Lutea,* 1902; *the Action of Mineral Acid on the Cellular Substance of Bacillus Typhosus,* 1004; *The Extraction of the Intracellular Toxin of the Colon Bacillus,* 1905; *The Split Products of the Tubercle Bacillus and Their Effects upon Animals, by Vaughan and Wheeler,* 1907; *A Study of the Chemistry of Bacterial Cellular Proteins,* 1909.

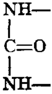
In 1922, Howard B. Lewis, who had been assistant and associate professor of physiological chemistry at the University of Illinois 1915-1922, was called to the professorship of physiological chemistry at Michigan. Lewis had his early training at New Haven, having taken the Ph.D. degree in physiological chemistry in the Sheffield Scientific

School in 1913. Active in research, he has worked in three distinct though related fields, studying the behavior of some *Hydantoin Derivatives in Metabolism, The Synthesis of Hippuric Acid in the Animal Organism,* and the *Metabolism of Sulphur.*

Studies by Lewis in 1912-1915 showed, contrary to what might be expected from the constitution of various hydantoin derivatives, that parabanic acid, for example, with its structure of

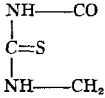


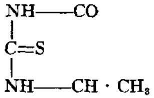
does not break down in dogs and rabbits into urea, and no toxic symptoms are developed by its administration. With hydantoin, experiments on cats, dogs and rabbits showed that the hydantoin nucleus is not destroyed in the body of these animals nor are any toxic effects manifested in opposition to the theory of the alleged toxicity of



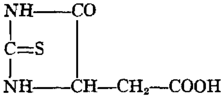
groups.

Similarly hydantoic acid, of which hydantoin is the cyclic anhydride, is not destroyed in metabolism when administered as the ethyl ester. Thiohydantoins, on the other hand, Lewis found are more or less toxic. Thus, 2-thiohydantoin and 2-thio-4-methylhydantoin





are both toxic to rabbits, in fatal doses causing albuminuria, the toxicity presumably being due to the replacement of the oxygen of the hydantoin nucleus by sulfur. Further, the sulfur contained in the 2-thiohydantoins is not oxidized in the body of the rabbit. On the other hand, 2-thiohydantoin-4-acetic acid



is not toxic in any ordinary dosage.

Referring to Lewis' studies on the synthesis of hippuric acid two papers may be noted: *The Synthesis of Hippuric Acid on a Glycocoll-free Diet,* 1914; *The Synthesis and Rate of Elimination of Hippuric Acid after Benzoate Ingestion in Man,* 1914. On a glycocoll-free milk diet the introduction of sodium benzoate causes no marked rise in the output of total nitrogen. The nitrogen eliminated as hippuric acid evidently comes from the nitrogen normally present as urea in the urine, since it was found that the urea decreased with increase in hippuric acid. This plainly implies that the "synthesis of glycocoll for the purposes of detoxication of the benzoate results from a deviation of the normal path of catabolism and not from a specialized metabolism." As an indication of the extent of the ability

of the human organism to detoxicate benzoic acid by conjugation with glycocoll and its elimination as hippuric acid, Lewis found that after a dose of 10 grams of sodium benzoate 85-90 per cent of the benzoate is excreted in 5 to 6 hours. During the period of greatest hippuric acid excretion after benzoate, it was observed that the urine had a lower content of urea and ammonia than in the normal or control period, showing again that the hippuric acid nitrogen originates in the nitrogen normally eliminated as urea.

Especially important were Lewis' studies of sulfur metabolism, twelve papers in all, a few of which may be cited: *The Relative Elimination of Sulphur and Nitrogen in the Dog in Inanition and Subsequent Feeding,* 1916; *The Influence of Small Amounts of Cystine on the Balance of Nitrogen in Dogs Maintained on a Low Protein Diet,* 1917; *The Relation Between the Cystine Content of Proteins and Their Efficiency in the Maintenance of Nitrogenous Equilibrium in Dogs,* 1920; *The Oxidation of Cystine in the Animal Organism,* with Lucie E. Root; *The Oxidation of Some Sulphur Compounds Related to Cystine in the Animal Organism,* with Robert M. Hill, 1924; *Can Taurine Replace Cystine in the Diet of the Young?* with George T. Lewis, 1926; *The Value of Diglycyl-cystine, Dialanyl-cystine, and Dialanyl-cystine Dianhydride for the Nutritive Requirement of the White Rat,* with George T. Lewis, 1927.

Among the many results recorded the following are worthy of note. The addition of small amounts of cystine to the diet of dogs on a low protein diet diminished the loss of nitrogen from the body and influenced favorably

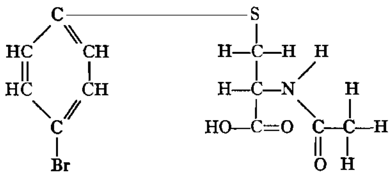
the nitrogen balance. Tyrosine and glycocoll, on the other hand, when added to the diet, under like conditions of experiment, failed to diminish the nitrogen loss, hence the conclusion seemed justified that there is a specific need for cystine in the metabolic processes, at least in the dog. Again, the same conclusion was indicated by the fact that casein with its low cystine content was found to be less effective in maintaining nitrogen equilibrium (in the dog) on low protein diet than serum albumin with its high content of cystine. If, however, casein was supplemented by cystine it was found to be as effective as serum albumin.

These facts obviously confirm the view that cystine is essential for maintenance and growth. On the other hand, neither taurine nor cysteinic acid can replace cystine entirely or in part for purposes of growth; a view which has been confirmed by William C. Rose and B. T. Huddlestun. Diglycyl-cystine and dialanyl-cystine, however, were found to promote growth in the absence of an adequate supply of cystine, but the dianhydride of dialanyl-cystine was not able to accomplish this.

Finally, the subcutaneous administration of the sodium salt of phenyluramino-cystine in rabbits gave results indicating that oxidation of the sulfur of the cystine molecule is connected with the process of deamination or the oxidation of the deamination products. In other words, if deamination of cystine be prevented by "blocking" the amino group (as in phenyluramino-cystine), the sulfur of the molecule is not oxidized normally, but is excreted in the urine in large part in the unoxidized fraction. In this connection it may be noted that Sherwin and his associates have shown that if a derivative of cysteine, with the

amino group protected from deamination, is fed to rabbits the cysteine is in part converted into cystine. This result, together with like results obtained by Lewis and others, point to the ready reversibility of the reaction cysteine ⇄ cystine.

The changes that cystine and its derivatives may undergo in the animal body have long been the subject of speculation and numerous investigators have turned their attention to the subject. Thus Carl P. Sherwin working with George J. Shiple has made a number of compounds of cystine, many of them never prepared before, with a view to studying their metabolism. Carl P. Sherwin is a graduate of Tübingen, Sc.D. 1915, assistant professor of physiological chemistry 1915-1917, professor since 1919 at Fordham University, New York, and his paper on *Some Derivatives of Cystine and Cysteine,* 1923, published in the *Journal of Biological Chemistry,* gives an interesting presentation of several new compounds, notably phenyl acetyl benzyl-cystine and p-bromophenyl mercapturic acid. This latter compound was prepared by feeding bromobenzene to dogs, the mercapturic acid separating from the urine in well-defined crystals, truly a good biochemical reaction.



*p-bromophenyl mercapturic acid*

Sherwin's experimental work has been both extensive and important, his contributions to the *Synthesis of Amino-Acids in the Animal Organism* and his numerous studies under the general title *Comparative Metabolism of Certain Aromatic Acids* having yielded results of great physiological value, especially as bearing on certain detoxication reactions in the body. In illustration, reference may be made to one study—the fifth in the latter series—entitled *Fate of Some Ring Substitution Products of Phenylacetic Acid in the Organism of the Dog, Rabbit and Man,* with Leopold R. Cerecedo, 1923.

On feeding *o*-nitrophenylacetic acid, *o*-aminophenylacetic acid, *o*-hydroxyphenylacetic acid, *o*-chlorophenylacetic acid and 2-4-dinitrophenylacetic acid, it was found that all five of these derivatives of phenylacetic acid are quite non-toxic physiologically. Two, *o*-aminophenylacetic acid and *o*-chlorophenylacetic acid, underwent detoxication, the others passing through the organism unaltered. This the authors considered indicated that *ortho* compounds are not easily oxidized in the organism and further that they are no more toxic than their isomeric *meta* and *para* derivatives. Moreover it appeared that these *ortho* compounds were less toxic than phenylacetic acid itself. In the detoxication of *o*-aminophenylacetic acid, conjugation occurred only in the rabbit "and this by the very unusual reaction of acetylation, adding thereby a new example to the few cases of this reaction already known." On the other hand, the rabbit excreted unchanged the *o*-chlorophenylacetic acid, while in the dog and man this acid was conjugated with glycocoll. This, the authors considered surprising, since it would have been expected that the

human organism would use glutamine in place of glycocoll as the detoxicating agent.

Referring again to the University of Michigan, mention should be made of the appointment of Henry C. Eckstein as assistant professor of physiological chemistry in 1926. A graduate of the University of Illinois, 1915, he had been trained in physiological chemistry at the Sheffield Scientific School, Yale, taking the Ph.D. degree in 1923, becoming then instructor in physiological chemistry at Michigan. His studies of human fat and related subjects, under the following titles, are especially worthy of note: *The Fatty Acids in the Subcutaneous Fat of Man,* 1925; *The Cholesterol and Phospholipid Content of the Cutaneous Epithelium of Man,* with Udo J. Wile, 1926; *The Cholesterol Content of Hair, Wool and Feathers,* 1927; *The Distribution of Some of the More Important Amino-Acids in the Globulin of the Thyroid Gland,* 1926; all published in the *Journal of Biological Chemistry.*

Ross A. Gortner, who has done extensive work on colloids and proteins, the chemistry of animal pigments and the chemistry of embryonic growth, has made a special study of *Sulphur in Proteins.* Educated at several university centers, M.A., Toronto, 1908, Ph.D., Columbia, 1909, connected with the division of agricultural biochemistry at the University of Minnesota, he has been professor of that branch of biochemistry since 1917. In a recent paper on *Derivatives of l- and i-Cystine,* with Walter F. Hoffman, 1926, he has given the results of an extensive study of the two forms of cystine, noting particularly that while they have the same empirical formula, they possess different melting points and are generally

unlike in crystalline form. Since *i*-cystine could not be resolved into optically active components he was led to believe that it was not racemic cystine but rather "an internally compensated form." Further, since the cystine prepared from calculi had an optical rotation of $[\alpha]_D = -242.6°$, which is 20° higher than the usual value for *l*-cystine, as prepared from protein by acid hydrolysis, it would appear probable that several isomers exist.

Gortner's recent book, 1929, *Outlines of Biochemistry*, a treatise on "the organic chemistry and the physico-chemical reactions of biologically important compounds and systems" is especially valuable in that it leads to a proper understanding of the part organic chemistry and physical chemistry play in living processes, special emphasis being laid on the study and investigation of the fundamental chemical and physico-chemical reactions which occur in the normal organism whether animal or plant.

At the University of Minnesota Jesse F. McClendon has been the professor of physiological chemistry since 1920, having been connected with the department of physiology for some years previous to that date. He has been especially interested in problems connected with the hydrogen-ion concentration, alkaline reserve and some phases of metabolism. His studies of the distribution of iodine in relation to goitre are particularly noteworthy. A graduate of the University of Texas, B.S., 1903, he pursued graduate studies at the University of Pennsylvania, being granted the Ph.D. degree in 1906.

Some of his more important pieces of work, carried on with various collaborators, are the following: *The Hydro-*

*gen-ion Concentration of the Contents of the Small Intestine,* 1918; *The Hydrogen-ion Concentration of Foods,* 1919; *Effect of Diet on the Alkaline Reserve of the Blood,* 1919; *Metabolism of Calcium and Phosphoric Acid on Isorachitic Diets,* 1922; *The Determination of Iodine in Food, Drink and Excreta,* 1924; *The Determination of Hydrogen Ions in the Gastric Contents,* 1924; *Colloidal Properties of the Surface of the Living Cell,* 1926; published in the *Journal of Biological Chemistry.* Other workers in physiological chemistry at the University of Minnesota will be referred to later in connection with the Mayo Foundation.

Turning to a different field of biochemical inquiry, the lipoids of the blood and fat metabolism, we come in touch with the work of Walter R. Bloor, professor of biochemistry in the School of Medicine at the University of Rochester. A graduate of Harvard University, Ph.D., 1911 and a pupil of Folin, he has been extremely active in research in the several biochemical laboratories he has been connected with; assistant professor at Harvard 1914-1918, professor at the University of California 1918-1922, and since 1922 at Rochester. Through the thoroughness of his work he has made himself an expert in the field of lipoid chemistry, especially in its relation to the blood.

Among his many contributions, the following are selected as indicative of the general character of his investigations: *Cholesterol and Cholesterol Esters in Human Blood,* with Arthur Knudson, 1916; *The Blood Lipoids in Nephritis,* 1917; *The Distribution of Phosphoric Acid in Normal Human Blood,* 1918; *Blood Phosphates in the Lipemia Produced by Acute Experimental Anemia in*

*Rabbits,* with E. D. Farrington, 1920; *The Lipoid Balance in the Blood,* 1921; *Lipemia,* 1921; *Determination of Fatty Acids (and Cholesterol) in Small Amounts of Blood Plasma,* with K. F. Pelkan and D. M. Allen, 1922; *Fat Excretion,* with Elsie Hill, 1922; *The Fatty Acids of Blood Plasma,* 1923; *The Distribution of the Unsaturated Acids of Blood Plasma,* 1924; *Distribution of Unsaturated Fatty Acids in Tissues,* 1926; all published in the *Journal of Biological Chemistry.*

The results of Bloor's various studies have thrown much light on the distribution of lipoids in the blood, both in health and disease, especially the compounds of phosphoric acid of the lecithin type; compounds which are probably of great importance in the metabolism of fat. Thus it has been suggested by Bloor that lecithin or a similar phospholipoid may be an intermediate step in the utilization of fat in animals, since it has been found that during fat absorption lipoid phosphorus increases in the blood. Moreover, accumulation of fat in the liver under various conditions is probably followed by transformation of a portion of the fat into a phospholipoid of that organ.

As Bloor's analyses show, the phosphoric acid compounds present in human blood are of two distinct classes: (1) the acid-soluble compounds, soluble in dilute acids and precipitated with the proteins by alcohol-ether, and (2) the lipoid-phosphoric acid compounds soluble in alcohol-ether and precipitated with the proteins by dilute acids. The corpuscles are relatively richer in all types of these compounds than the blood plasma. In the second of the above groups, substances of the type of lecithin are

conspicuous. Especially noticeable was the fact that in normal human blood there is a constant relation between the amounts of free cholesterol and cholesterol esters. Thus in whole blood the average percentage of cholesterol in combination as esters was found to be 33.5 per cent, in plasma 58 per cent of the total cholesterol.

In pathological conditions, the relations between free and combined cholesterol was found to be normal in all cases except in carcinoma and nephritis, where the percentage combined as ester was low. The general constancy of the relationship between free and bound cholesterol would seem to indicate that with cholesterol esters, as with other lipoids, there is an efficient regulation, that the cholesterol plays an important part in fat metabolism and "that the metabolic habits of the individual are not easily upset even by severe disease."

Regarding the relationship between lecithin, cholesterol and fat in normal blood, Bloor found that it was constant within narrow limits for the individual and within wider limits for the species. While fat is being absorbed there may be a disturbance of this balance, but as a rule equilibrium is quickly regained either by elimination of the disturbing constituent, or by raising the level of the several lipoids until the balance is established at a higher level. Whenever there are marked disturbances, cholesterol and lecithin especially are prone to maintain a constant relationship to each other while fat is much more inclined to be variable.

As Bloor has stated, "there appears to be a threshold both in amount and time within which changes may occur in one of the lipoid constituents without disturb-

ing the others. Thus, there may be a moderate change of fat alone for a short time without any change in lecithin or cholesterol. A greater change or longer lasting change in fat will result in changes in the lecithin, while cholesterol appears to be less readily affected by changes in fat." In lipemia, of whatever origin, it would appear that all three blood lipoids are increased in amount, fat usually showing the greatest ultimate increase and cholesterol next. In the words of Bloor "generally there is a sequence in the appearance and disappearance of the three lipoids, fat being the first to increase, lecithin next and cholesterol last, while during the clearing up of the lipemia fat diminishes first and the cholesterol last."

"High values for lecithin and cholesterol often persist for some time after the fat has reached approximately normal values. In most instances the values for the ratio $\frac{\text{lecithin}}{\text{cholesterol}}$ are markedly below normal, due to the greater increase of cholesterol over lecithin." While the fat which produces lipemia may have an exogenous or endogenous origin or both, it is to be noted that the phenomena of the lipemia are the same in either case. As to the excretion of fat, Bloor found that when moderate amounts of fat are ingested, the fat of the feces is in large measure independent of the diet, its composition being closely akin to that from a fat-free diet. This comparative constancy in the composition of the fat of the feces naturally lends favor to the view of a fat excretion from the intestine.

Bloor's investigations of the distribution of lipoids in the blood in various diseases have given interesting results, but only a brief reference can be made here. In

acute experimental anemia in rabbits, it was found that of the phosphoric acid compounds of the blood, the lipoid phosphorus was most noticeably affected by the lipemia which resulted. Values as high as five times the normal were observed in the plasma, and twice or over the normal value in the corpuscles. It was also noted that when the lipoid phosphorus was at these higher levels in either plasma or corpuscles, there was an accompanying increase in inorganic phosphate, which would seemingly imply some relationship between inorganic phosphorus and the lipoid phosphorus.

Equally interesting was the fact that in the newly formed blood corpuscles the lipoid phosphorus was noticeably higher in amount than in the older corpuscles, this being the only form of phosphorus which showed increase in the new cells. Whether this increase is to be attributed to an effect of the lipemia or is merely a characteristic of the younger corpuscles was not determined. In nephritis, there was a distinct retardation of fat assimilation, due, as Bloor considered, probably to the decreased alkalinity of the blood and tissues. Analysis of the blood showed a high content of fat in both plasma and corpuscles, together with a high content of lecithin in the corpuscles. Cholesterol values, on the other hand, were practically normal.

Regarding the unsaturated fatty acids, Bloor found abundant evidence of the presence of highly unsaturated fatty acids in normal (fasting) blood plasma. The data collected showed that the fatty acids in the plasma are present "in definite compounds of which the composition with respect to saturated and unsaturated fatty acids is

constant and which exist in definitely balanced relation to each other." These unsaturated acids are present mainly in combination with cholesterol, the esters, however, consisting not only of palmitate and oleate, but also of a large proportion of the esters of the more unsaturated acids. Bloor considers that in the study of fat metabolism serious attention must be given to the rôle of cholesterol, since it has apparently an important part to play.

Finally, attention must be called to the presence of unsaturated fatty acids in the tissues. Working with the muscle tissue of the heart (beef) Bloor found that the largest proportion of unsaturated acids was contained in the cephalin fraction, the next largest in the lecithin fraction. The total unsaturation he found was in the same order, the iodine value of the liquid acids being on an average 170 for cephalin, 148 for lecithin and 100 for the fat fractions. The most important unsaturated acids were oleic, linolic and arachidonic acid. Cholesterol esters were absent from heart muscle.

At the University of Wisconsin, Harold C. Bradley has been connected with the development of physiological chemistry since 1906, when he was appointed assistant professor. A graduate of the University of California in 1900, he studied physiological chemistry in the Sheffield Scientific School at Yale, being granted the Ph.D. degree in 1905. Going to Wisconsin in 1906 he served as assistant professor until 1913, when he was made associate professor and in 1917 professor of physiological chemistry. His experimental work has touched several fields, but most interest centers on his studies of autolysis.

Among the many papers from his laboratory on th

subject the following may be cited: *The Accelerating Effect of Manganous Chloride on Liver Autolysis,* with Max Morse, 1915; *The Acceleration of Liver Autolysis,* 1915; *Acceleration of Liver Autolysis,* with Joseph Taylor, 1916; *The Latent Period in Autolysis,* with Joseph Taylor, 1916; *The Influence of Bile in Autolysis,* with Joseph Taylor, 1917; *Effect of Certain Colloids upon Autolysis,* with H. Felsher, 1920; *The Nature of Autolytic Enzymes,* 1922; *Hydrogen-Ion Concentration in Autolysis,* with E. L. Sevringhaus and A. E. Koehler, 1923; *Experimental Atrophy of Muscle Tissue,* with K. K. Chen and Walter Meek, 1924; *The Acids Formed in Autolyzing Liver,* by Elmer L. Sevringhaus, 1923; *The Phosphoric Acid Liberation in Liver Autolysis,* by Elmer L. Sevringhaus, 1923, all published in the *Journal of Biological Chemistry.*

The problem of autolysis as witnessed in many tissues and organs of the body has been the subject of various investigations in many laboratories without, however, satisfactory explanation of all the results obtained. Bradley's work and that of his associates has contributed much to an understanding of the conditions which influence autolytic changes and has thrown light upon the character and extent of the processes involved. In his earlier experiments Bradley found that under ordinary conditions the liver of pigs and dogs autolyzed 25 per cent of the total nitrogen of the tissue, 75 per cent remaining undigested although the enzyme was still active for a long time after the reaction came to approximate equilibrium. The addition of manganous chloride to the autolyzing tissue so changed the reaction that from 75 to 90 per cent of the nitrogen was converted into soluble products, not precipitable by

:annic acid. Among the many findings reported by Brad-ey the following may be cited:

Apparently, all soluble salts of manganese increase liver autolysis, due, as Bradley believed, to an alteration of the normally resistant fraction of the liver proteins, by which they become digestible by the protease. He found further that foreign proteins, such as casein and peptone, as well as the coagulated liver proteins, are digested by the autolytic enzymes of the liver, but this was not true of the crystallized vegetable protein edestin, unless manganous chloride was present. Ovalbumin, on the other hand, could not be digested by the liver enzymes except in the presence of hydrochloric acid.

A very low concentration of the hydrogen ion is sufficient to induce alteration of the undigestible liver proteins by which they become digestible. A 0.02 M hydrochloric acid gives marked acceleration. Progressive changes in the reaction of the autolyzing liver from an alkalinity expressed by pH = 7.4 to the optimum acidity of pH = 6.0 (measured in the dialysate) cause proportionate changes in the rate and extent of autolysis from nearly zero to 90 per cent digestion. Such changes in the reaction of the tissue alter the mass of digestible protein present in the liver from nearly zero, when alkaline, to nearly complete digestibility at an acidity of pH = 6.0.

Bradley considers that many of the phenomena of autolysis in the body, such as atrophy, necrosis, involution, etc., probably depend upon some such mechanism as the above. In such tissues as are altered by a low acid concentration, in the same sense as in the liver, the development of such acidity may have two effects: (1) remove the in-

hibitory alkalinity and (2) alter the proteins, with a resulting increase in the mass of substratum. Hence, as Bradley believed, autolysis is not due to autocatalytic action but the process, certainly in its extent, depends upon the condition of the substratum. "The tissue grows more distinctly acid to litmus as it autolyzes. Accompanying the increased acidity and caused by it, is a corresponding change in the protein substratum of the tissue. If much acid is produced the extent of digestion will be large, if little is produced digestion will reach equilibrium at a low level."

Again, it is the mass of the substratum available for hydrolysis by the proteases normally present in the liver cells that determines the length of the latent period, the rate of proteolysis as measured by concentration of products, and the final equilibrium of the digestion. The time required for the appearance of measurable amounts of amino-acids is greater than that required for increase in non-coagulable nitrogen; the so-called latent period is merely the lag between the initial stages of proteolysis and the final products.

As has been shown by various workers, tissues immersed in bile or its salts undergo rapid cytolysis—within a few hours—and the view has been expressed that this cytolysis is produced by virtue of the co-enzyme or activating action of the constituents of the bile on the autolytic enzymes or processes. Bradley, however, found that bile does not activate the enzymes associated with the phenomena of autolysis, nor does it act as a co-ferment. Further, autolysis does not parallel the rapid cytolysis of tissues immersed in bile or its salts, and bile does not

accelerate the autolysis of liver, spleen, kidney, thymus and heart muscle to any significant degree. The cytolytic effect of bile on these tissues Bradley considers to be quite distinct from the process of autolysis.

Again, it has been claimed by several earlier investigators that colloids such as silver sols, arsenious sulfide and colloidal ferric hydroxide accelerate autolysis, the opinion being held that substances of this order may activate the enzymes of the liver, or they may counteract some anti-autolytic substance in the autolyzing mixture or they may have a direct catalytic action thereby facilitating hydrolysis of the proteins. Bradley's experiments, however, have led to the conclusion that whenever such substances show any accelerating effect acidity can also be detected; purified by long dialysis they failed to show accelerating action.

As to the nature of the enzymes involved in tissue autolysis Bradley emphasizes the presence of an ereptic type of enzyme which converts the primary cleavage products of protein to amino-acids but which has no action on the native tissue proteins. This enzyme complex he found to be active between the hydrogen-ion levels of pH 8 to 3— and was present in the tissues in abundance. It was completely inactive at pH 1+. Pepsin and trypsin as enzymes of the autolytic complex were not found. There was evidence, however, of an enzyme complex which digests acid-salts of the tissue proteins between pH 7 and 3. It is completely inhibited at an hydrogen-ion level of pH 2.6, while pepsin remains active in an hydrogen-ion concentration of pH 1±. This, Bradley termed the ***primary protease*** of the tissue. "The action of this enzyme constitutes the

limiting factor in the autolytic machinery and its activity is in turn conditioned by the amount of acid produced."

Following death, Bradley found that the hydrogen-ion concentration of liver cells increased with "almost explosive rapidity," a maximum of pH 6 being reached in 4 to 48 hours. Addition of alkali caused a very rapid increase of the hydrogen ion, sufficient even to change the pH from 9 to 7+ in 24 to 48 hours. Phosphoric acid apparently accounts for the larger part of the increasing hydrogen-ion concentration of both the control and alkaline breis. Fatty acids are likewise produced in the autolyzing liver and by competing with the proteins for the basic groups contribute to the increased hydrogen-ion concentration.

Any consideration of physiological chemistry at the University of Wisconsin would be far from complete without reference to the work of Edwin B. Hart and Harry Steenbock, professors of agricultural chemistry, whose studies of animal nutrition, protein decomposition products, and vitamines have brought to them well-deserved recognition. To some of their accomplishments reference will be made in a later chapter.

# CHAPTER VII

Chemistry of the brain, Chittenden and Frissell, William J. Gies, Waldemar Koch, Levene and Jacobs—At the University of Chicago, Albert P. Mathews, Fred C. Kocb, Martin E. Hanke, A. Baird Hastings, Rollin T. Woodyatt, Esmond R. Long, Milton T. Hanke and Karl K. Koessler, H. Gideon Wells, Florence B. Seibert—Studies on the chemistry of anaphylaxis, Wells, Wells and Osborne—Work of Victor C. Vaughan, University of Michigan.

During my early years, at a time when chemico-physiological research had little recognition in this country, the yearly "blue books" from London containing the *Reports of the Medical Officer of the Privy Council and Local Government Board,* dealing with the public health and especially with "the chemical identification of disease," were eagerly sought and read with interest. The chemist who was largely responsible for the many scientific studies appearing in these books was J. L. W. Thudichum and from 1868 through many years following he reported the results of his numerous investigations, notably on the chemistry of the brain of both man and animals.

At that date there was great interest in the so-called brain wax or brain fat, and Thudichum with the abundant facilities at his command and with quantities of material far beyond the amount available for the unofficial investigator did much to broaden knowledge regarding the lecithins, kephalins, myelins, cerebrin, phrenosin, kerasin,

cerebric acid or protagon, concerning which there was much diversity of opinion. The cerebric acid of Frémy or the protagon of Liebreich occupied an anomalous position and Thudichum among others held the view that protagon was probably proximately composed of cerebrin and lecithin. Later, in 1901, Thudichum published at Tübingen a book under the title *Die chemische Konstitution des Gehirns des Menschen und der Thiere*, giving his views at that date regarding the chemical structure of the brain. The work of Thudichum in this field constitutes a background of knowledge upon which many investigations have since been based.

The phosphorized constituents of nervous tissue have long been the subject of controversy, ever since the discovery of protagon by Liebreich in 1865, Diaconow in 1868 claiming that the substance was in reality a mixture of cerebrin and lecithin. Gamgee and Blankenhorn, 1879, however, by careful work obtained results apparently confirming Liebreich's views, while Kossel and Freytag, 1893, came to the conclusion that there were several protagons, their results rather suggesting the composite nature of the substance. Further, Wörner and Thierfelder, 1900, likewise concluded from their experiments that protagon is either not an individual substance or if so it is extremely labile, both chemically and physically. Thudichum, however, had separated from the brain a number of phosphorus-containing substances which he classified under the three heads of lecithins, kephalins, and myelins, thus adding to the complexity of the situation and giving strength to his view that the so-called protagon was not a chemical

individual but a mixture of one or more phosphorized substances with cerebrin or other like bodies.

In this country Chittenden and Frissell, 1897, investigated the phosphorus-containing substances of the brain with results that led to the conclusion that protagon contains only a small proportion of the total phosphorus of the tissue and that other phosphorized organic substances, such as lecithins, exist preformed in the tissue in relatively large proportion. Further, it was found that, contrary to the prevalent opinion, the so-called protagon by repeated fractional crystallization from 85 per cent alcohol constantly lost phosphorus, indicating quite clearly that an alcohol-soluble body richer in phosphorus than the original protagon was being separated and pointing to the view that protagon in reality was quite unstable, or indeed that it might be made up of a mixture of several substances, loosely combined.

This subject of protagon has been studied quite extensively by Gies and his associates in the laboratory of physiological chemistry in the College of Physicians and Surgeons, Columbia University, the more conspicuous contributions being *Notes on the Protagon of the Brain* by W. W. Lesem and William J. Gies, 1902; *Is Protagon a Mechanical Mixture of Substances or a Definite Chemical Compound?* by Edward R. Posner and William J. Gies, 1905; *On the Chemical Nature of Paranucleoprotagon, a New Product from Brain,* by Mathew Steel and William J. Gies, 1907; *Further Observations on Protagon,* by William J. Gies, 1907.

The work of Gies and his associates all tended to show that protagon is an indefinite mechanical mixture of

various brain substances more or less dissimilar in their content of phosphorus and sulfur. They also inclined to the belief that phrenosin, pseudocerebrin and cerebron are identical substances. Further, the view advanced by Ulpiani and Lelli (1902), that all the protagon obtainable from brain tissue occurs there in combination with paranuclein as paranucleoprotagon, Steel and Gies found to be untenable. They could obtain no evidence that the so-called paranucleoprotagon is a definite individual substance, but rather like protagon itself is a mixture of several substances more or less readily split apart by appropriate treatment with alcohol.

In 1912 Waldemar Koch of the University of Chicago reported as the result of his experiments that the so-called protagon is made up of a phosphatide containing choline, a cerebroside-containing sugar, a complex combination of a choline-free phosphatide with a cerebroside to which an ethereal sulfuric acid group is attached, thereby furnishing additional evidence that the term protagon cannot have any significance.

Waldemar Koch was a graduate of Harvard University, B.S., 1898, Ph.D., 1900, assistant professor of physiological chemistry and pharmacology at the University of Missouri 1903-1906, associate professor of pharmacology at the University of Chicago from 1908 until his death in 1912. He was especially interested in methods of quantitative study of animal tissues, particularly of the nervous system. During the brief period of his active life he accomplished much and the character of his accomplishments gave promise of still greater usefulness for the future had his life been spared.

In his work on the brain, the following topics are to be emphasized: *Methods for the Quantitative Chemical Analysis of the Brain and Cord,* 1904; *The Quantitative Estimation of the Lecithans,* with Herbert S. Woods, 1905; *A Preliminary Study of the Chemistry of Nerve Tissue Degeneration,* with William H. Goodson, 1906; *Should the Term Protagon Be Retained?* 1912; *Contributions to the Chemical Differentiation of the Central Nervous System,* with Mathilde L. Koch, this last being a series of four papers, the experimental work having been conducted in the Hull Laboratory of Biochemistry and Pharmacology at the University of Chicago, the Wistar Institute of Anatomy and Biology at Philadelphia and the Psychiatric Institute, Ward's Island, New York. The third paper of this series was entitled *The Chemical Differentiation of the Brain of the Albino Rat During Growth,* 1913, and contains results of special physiological significance.

The most marked and characteristic chemical changes during growth, as pointed out by the authors, are correlated with the anatomical differentiation in the brain. Among the chemical changes noted were the following: a diminution in the relative percentage of protein in the total solids due to the formation of a large amount of lipoid matter, the lipoids which appear coincident with the medullation being the cerebrosides and the sulfatides and present mainly in the medullary sheaths; a great outburst of phosphatide formation at the very beginning of medullation, although the phosphatides are present also in large amounts in both the cells and sheaths before medullation; a great increase of colloidal matter, coincident with

the slowing up of the metabolic processes characteristic of senescence, composed largely of relatively inactive, supporting structures; decrease of extractives, these being present in largest amounts during fetal and early life when growth and metabolism are at a maximum. The following table, taken from Koch's paper, gives a clear expression of the changes in chemical composition noted at different ages:

CHEMICAL COMPOSITION OF THE BRAIN OF THE ALBINO RAT AT DIFFERENT AGES

*Constituents in Per Cent of Solids*

| *Age in Days* | *1* | *10* | *20* | *40* | *120* | *210* |
|---|---|---|---|---|---|---|
| Proteins | 58.3 | 56.4 | 52.7 | 48.7 | 48.0 | 48.5 |
| Phosphatides | 15.2 | 12.3 | 21.7 | 20.5 | 21.3 | 22.0 |
| Cerebrosides | * | * | 2.9 | 6.3 | 8.4 | 8.4 |
| Sulphatides | 1.5 | 2.6 | 2.6 | 2.7 | 3.6 | 4.5 |
| Organic extractives and Inorganic constituents | 16.5 | 15.1 | 15.3 | 13.8 | 9.8 | 9.8 |
| Cholesterol (by difference) | 9.0 | 13.5 | 4.8 | 8.0 | 8.9 | 6.8 |
| Total sulphur | 0.96 | 0.83 | 0.70 | 0.58 | 0.57 | 0.58 |
| Total phosphorus | 1.82 | 1.48 | 1.67 | 1.55 | 1.44 | 1.39 |
| *Distribution of sulphur in per cent of total sulphur* | | | | | | |
| Protein sulphur | 30.0 | 44.2 | 55.3 | 62.4 | 61.2 | 63.8 |
| Lipoid sulphur | 2.8 | 6.1 | 7.5 | 10.1 | 12.8 | 15.6 |
| Neutral sulphur | 47.3 | 45.4 | 27.5 | 19.3 | 19.2 | 14.5 |
| Inorganic sulphur | 19.9 | 4.3 | 9.7 | 8.2 | 6.8 | 6.1 |
| *Distribution of phosphorus in per cent of total phosphorus* | | | | | | |
| Protein phosphorus | 13.3 | 13.9 | 6.0 | 9.9 | 7.4 | 6.8 |
| Lipoid phosphorus | 33.2 | 36.1 | 52.2 | 56.1 | 65.8 | 67.6 |
| Water-soluble phosphorus | 53.5 | 50.0 | 41.8 | 34.0 | 26.8 | 25.6 |

* Presumably none present at birth and ten days.

In his elaborate studies bearing on the chemical analysis of the brain, Koch devised methods for the quantitative determination of the various substances isolated at that date, 1904, so far as possible, such as the simple proteins, mostly globulins; nucleoproteins; neurokeratin; water-soluble extractives; the lecithans or phosphatides including the lecithins, kephalins and myelins; the cerebrins including phrenosin, kerasin and cerebrin acids; cholesterol, etc. The following table taken from Koch's paper shows the main points of distinction in chemical composition between the grey and white matter of the human brain.

CHEMICAL COMPOSITION OF HUMAN BRAIN (EPILEPTIC)
Results Calculated in Per Cents of Solids

| | *White Matter Corpus Callosum* | *Grey Matter Cortex (prefrontal)* |
|---|---|---|
| Simple proteids ...... | 10.00 | 27.71 |
| Nucleoproteids ...... | 11.56 | 16.66 |
| Neurokeratin ........ | 8.40 | 2.22 |
| Extractives ......... | 4.75 | 8.78 |
| Lecithines .......... | 16.22 | 17.44 |
| Kephaline, myeline ... | 10.91 | 4.11 |
| Phrenosine, kerasine .. | 14.29 | 8.61 |
| Cholesterine ........ | 15.20 | 3.89 |
| Sulphur compound.... | 4.37 | 8.06 |
| Total solids ..... | 32 per cent | 18 per cent |

Finally, it should be added that Koch's analyses of nervous tissue in cases of general paralysis of the insane and in experimental degeneration produced by cutting the cord of a dog and allowing it to degenerate for nearly two weeks led to the general conclusion "that the nervous

system more than any other tissue, both in pathological and experimental degeneration, tends to keep its relative composition constant, which observation is in harmony with the results obtained in starvation."

Regarding the exact chemical nature of the organic constituents of nervous tissue, Phoebus A. Levene of the Rockefeller Institute for Medical Research and his coworkers have devoted considerable time to the study of the cerebrosides and phosphatides of brain tissue, with special reference to their chemical structure, concerning which there has long been disagreement. Various studies have appeared from their laboratory at frequent intervals dealing with some aspect of the problem, their results throwing more or less light upon the chemical relationship of the many substances that enter into the make-up of these two classes of compounds. Thus, in 1912, appeared a paper *On Cerebronic Acid,* with W. A. Jacobs, in which it was shown that this acid prepared by the hydrolysis of cerebrin is the normal α-hydroxypentacosanic acid, occurring in two isomeric forms, one optically inactive and the other dextrorotary, $[a]_D^{30°} = +4.16°$. The hydrocarbon of cerebronic acid is apparently $C_{25}H_{52}$, the acid value for the pure acid corresponding to a molecular weight value of $C_{25}H_{50}O_3$.

The same year appeared another paper by Levene and Jacobs, *On the Cerebrosides of the Brain Tissue,* in which the attempt was made to establish the relationship, or points of distinction, between phrenosin, cerebrin, cerebron and kerasin. The individuality of these cerebrosides has long been considered by many writers as questionable, for the exact relationship of one to another had never

been clearly established. As Levene has pointed out, there is unanimity of opinion regarding the identity of the base and of the carbohydrate present in the four cerebrosides, consequently such differences as exist must depend upon the character of the fatty acids that enter into the structure of their molecules. Devoting their attention therefore to a study of the acids obtainable from the several cerebrosides, they were soon led to the conclusion that only a substance having the composition of cerebronic acid was to be found among their decomposition products, the acid, however, always possessing a low melting point. It was then discovered that this acid was the optically inactive cerebronic acid.

Hence it would seem to follow that the above cerebrosides are alike in the character of their components, the minor differences in reaction such as solubility and optical activity, depending, it may be, upon the proportion of optically active and inactive substances. Levene inclined to the view that the three cerebrosides are all mixtures of stereoisomeric substances and he suggested that it would be simpler to abandon most of the old nomenclature and to designate the cerebrosides as *d*-cerebrin, *l*-cerebrin and *dl*-cerebrin. Again, as he states, cerebrin, cerebron and phrenosin correspond to *d*-cerebrin; kerasin and homocerebrin to *dl*-cerebrin. In a later paper, 1913, employing different methods of treatment of the brain tissue, Levene reported the identification of an acid having the composition $C_{24}H_{48}O_2$, lignoceric acid (?), as among the decomposition products of the cerebrosides, and he considered it necessary to defer decision as to the "existence of isomeric cerebrosides of cerebronic acid

until it will be possible to separate completely cerebrosides of cerebronic acid from the other cerebrosides."

In this connection it is to be noted that Levene and Jacobs, 1912, obtained by hydrolysis of cerebrin a substance originally discovered by Thudichum, through hydrolysis of phrenosin, *viz.*, sphingosin. This body, Levene ascertained to be an unsaturated mono-amino-dihydroxy-alcohol, with two hydroxyl groups in the molecule, but with uncertainty as to the position of the double bond and of the hydroxyl groups. Sphingomyelin Levene found, 1913, yields on hydrolysis an acid having the composition $C_{24}H_{48}O_2$, with a melting point of 81° C. and which forms an ethyl ester melting at 55°-56° C., *i.e.*, lignoceric acid. Again, by the action of potassium permanganate on cerebronic acid in alkaline solution, a similar acid was obtained.

Like cerebronic acid, lignoceric acid was found to have a normal carbon chain, and from the reactions obtained it appeared to be a normal acid of twenty-four carbon atoms. Methyl and ethyl esters were prepared with the acid obtained from cerebronic acid, both showing the melting points of the corresponding esters of lignoceric acid. Other studies on the cerebrosides, by Levene and his co-workers, have furnished additional data of chemical value which, however, cannot be referred to here.

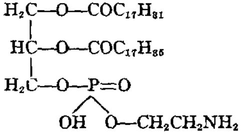
Regarding the phosphorized constituents of the brain, particularly the lecithins and the kephalins, there had long been need of a careful study of their chemical structure by modern methods, since a large part of the current knowledge concerning them was based on observations lacking that accuracy which present-day methods in or-

ganic chemistry are able to furnish. Again, the older processes of preparation and separation were not wholly adequate to insure purity of products, hence doubt had arisen regarding the true chemical relationship of these phosphorized lipoids. To a better understanding of these matters Levene and his associates have contributed much, as the following list of studies will indicate: *Hydrolecithin and its Bearing on the Constitution of Cephalin,* with C. J. West, 1917; *Preparation of Pure Lecithin; Composition and Stability of Lecithin Cadmium Chloride,* with C. J. West, 1918; *The Bearing of Cuorin on the Structure of Cephalin,* with S. Komatsue, 1919; *The Glycerophosphoric Acid of Cephalin,* with Ida P. Rolf, 1919; *Lecithin of the Brain,* with Ida P. Rolf, 1921; *Unsaturated Fatty Acids of Brain Lecithins,* with Ida P. Rolf, 1922; *Unsaturated Fatty Acids of Brain Cephalins,* with Ida P. Rolf, 1922; all published in the *Journal of Biological Chemistry.*

From these studies it appears that cephalin as it had been obtained in the past was in reality a mixture of true cephalin and of all the products of its intermediary hydrolysis, namely, monostearyl-glycerophosphoric aminoethanol ester, monostearyl-glycerophosphoric acid and glycerophosphoric acid. Cuorin is apparently an individual phosphatide differing from both lecithin and cephalin; differing from cephalin in that it contains a larger proportion of lower fragments of cephalin.

Again, Levene has definitely determined that the glycerol obtained by hydrolysis of cephalin is linked directly to the phosphoric acid and not indirectly, as some investigators have claimed, through attachment of the

glycerol to the aminoethanol. In other words he has furnished proof that glycerophosphoric acid enters into the structure of the cephalin molecule and that the acid is identical with that present in lecithin, having the same slight levorotation, and not an optical isomer of it. Consequently, the structural formula of cephalin is apparently definitely fixed.



There are undoubtedly different forms of lecithin, the differences presumably being dependent upon the character of the fatty acids in the molecule. Thus, Levene has found that while the lecithin of brain tissue and the lecithin of the egg yolk are alike in containing oleic, stearic and palmitic acids, the lecithin from liver tissue yields an unsaturated acid of the linolic series in place of oleic acid. Further, the lecithin from liver contains of the saturated acids only stearic acid. Moreover, Levene has found of the unsaturated fatty acids present in both brain lecithins and cephalins, acids with more than one double bond. Of these, arachidonic acid was isolated. There is, however, much still to be learned regarding the brain lipoids.

At the University of Chicago, physiological chemistry has flourished for many years in an atmosphere especially favorable for its development. With Jacques Loeb and A. J. Carlson in general physiology, Frank R. Lillie in ex-

perimental zoölogy, John U. Nef and Julius Stieglitz in organic chemistry, and H. Gideon Wells in pathology, there was a favorable environment which helped materially in the growth of physiological chemistry at that educational center. Further, the university was fortunate in having as its active worker in the science Albert P. Mathews from 1901 to 1919, professor of physiological chemistry during the last fourteen years of that period. In 1919 he went to the University of Cincinnati to take charge of the physiological chemistry there.

Mathews was a graduate of the Massachusetts Institute of Technology, 1892, a student with Kossel at Marburg 1895-1897, Ph.D. of Columbia University 1898. His work at the University of Chicago has covered a wide range of subjects, such as the physiology of secretion, nature of toxic and antitoxic action, chemical relations of nerves, the potential of ions and pharmacological action, protoplasmic respiration, etc. Among his earlier studies from the Hull physiological laboratories, the following may be referred to: *The Relation Between Solution-Tension, Atomic Values and the Physiological Action of the Elements*, 1904; *The Cause of the Pharmacological Action of the Iodates, Bromates, Chlorates, Other Oxidizing Substances and Some Organic Drugs*, 1904; *The Toxic and Anti-toxic Action of Salts*, 1905; *The Nature of Chemical and Electrical Stimulation—The Tension Coefficients of Salts and the Precipitation of Colloids by Electrolytes*, 1905; published in the *American Journal of Physiology.*

In the first of these experimental studies the object in view was the correlation of the physiological action of

the elements with their chemical or physical properties. Working with Fundulus eggs as a type of protoplasm, Mathews arrived at the conclusion that the physiological action of any cation or anion varies inversely with the solution tension. "Those ions with a very low solution tension are very poisonous; those with a high tension are relatively inert." From this it follows that the physiological action of a salt is a function of both ions and will vary inversely with the sum of the solution tensions of the ions, that is, with the decomposition tension of the salt. The antitoxic action of a salt likewise involves both ions.

Mathews also found that there was no definite relationship between the toxicity of a salt and the valence of either ion. Again he pointed out that there is an inverse relationship between the atomic volume and the poisonous action, while a direct relationship exists between equivalent weights and toxicity. Alteration in the permeability of the cell membranes must likewise be a responsible factor in toxic and antitoxic action; even a slight change in permeability can readily affect the rate or degree of such action.

In studying the precipitation of colloids, such as egg albumin, by electrolytes, Mathews arrived at the conclusion that the precipitating power of an electrolyte is determined primarily by the solution tension of the ions. The ion of opposite sign to the colloid precipitates; the ion of the same sign dissolves the colloid and is not inert. While the valence of the ion of the same sign as the colloid is of little or no importance in determining the action of the ion, the valence of the ion of the opposite sign to

the colloid is of importance. As Mathews stated, "if the valence is greater than unity, the effect of the polyvalence is to increase the precipitating power of the salt; the ion acts as if its solution tension was lower than it is." The importance of the valence of the polyvalent precipitating ion over a univalent salt of the same tension coefficient lies in the fact that such an ion can combine with several colloidal particles, thus forming larger aggregates. Valence is thus able to induce flocking by influencing the mass rather than the surface energy of the colloidal particles.

In another series of investigations from the Laboratory of Biochemistry and Pharmacology of the University of Chicago, under the general title of *The Spontaneous Oxidation of some Cell Constituents,* Mathews undertook a study of the power of certain substances to take up oxygen from the air under varying conditions with a view to throwing light on the reason why a substance, like sugar for example, oxidizes more rapidly in living tissues than it does outside the body.

The following papers bearing on this general subject have come from his laboratory: *The Spontaneous Oxidation of the Sugars,* 1909; *The Spontaneous Oxidation of Cystein,* with Sydney Walker, 1909; *The Action of Cyanides and Nitriles on the Spontaneous Oxidation of Cystein,* with Sydney Walker, 1909; *The Spontaneous Oxidation of Cystin and the Action of Iron and Cyanides upon It,* with Sydney Walker, 1909; *The Action of Metals and Strong Salt Solutions on the Spontaneous Oxidation of Cystein,* with Sydney Walker, 1909.

Mathews found that the sugars levulose, galactose, glucose, maltose and lactose all oxidized rapidly in the air

provided their solutions were alkaline. If, however, the solutions were neutral or acid oxidation did not occur. In the alkaline solution levulose oxidized most rapidly, the other sugars oxidizing only one-fourth as rapidly under like conditions. Further, with all the sugars there was an acceleration in the rate of oxidation and decomposition as the reaction proceeded, due, Mathews considered, to accumulation of several reducing molecules formed in the decomposition of the sugar, which may activate by dissociating the reducing substances.

The fact that glucose, for example, will not oxidize in the open air in a neutral solution Mathews believed due not to lack of active oxygen, but mainly to the fact that oxidation cannot take place until the sugar undergoes dissociation. In an alkaline solution, on the other hand, a salt of the sugar is formed, and this salt ionizes with a resultant disturbance of electrical equilibrium in the anion which leads to the decomposition and easy oxidation of the sugar.

In other words, the glucose molecule must be activated before it will burn. "Activation is brought about by a preliminary ionization in the alkaline solution, this ionization resulting in an upset of electrical equilibrium and a resulting molecular rearrangement leading to decomposition of the molecule and the oxidation and condensation of the dissociated parts," in harmony with the views advanced by Nef. Mathews considers it possible that a like process occurs in living matter, some substance being present which combines with the glucose molecule and causes its dissociation, the sugar then being burned in part, and in part converted into its several metabolic

products. The failure of living matter to burn glucose might thus be due not to the absence of oxidases but to the loss of power to dissociate the sugar.

Again, Mathews has suggested that two distinct groups of substances have been confused under the name "oxidases"; a group which hastens oxidation by action on the oxygen, activates the oxygen; and a group more important, specific in their affinities, which accelerate oxidation through action on the reducing substances and thereby increase their reducing power. These "reductases," since they may be responsible not only for the oxidations but for the various other more important metabolic activities of body protoplasm, Mathews has suggested might be termed "metabolases," a group which activates oxidation by dissociating the reducing substances.

Cysteine, Mathews found, oxidizes spontaneously with atmospheric oxygen at 20°-22° C. at a rapid rate, passing over into cystine. Here too the oxidation is sensitive to the reaction of the medium, the maximum speed being at a concentration in the neighborhood of $N^{10^{-8}}$, about the reaction of blood. "In this susceptibility and the position of the optimum, cysteine oxidation resembles protoplasmic oxidation." The explanation given by Mathews of this susceptibility to reaction is that "both the negative and positive cysteine ions are stable and that it is only the neutral non-ionized molecule which oxidizes rapidly, probably owing to the opening up of residual valences of the sulphur, or the greater ease of detachment of the hydrogen under these conditions."

Very small amounts of potassium cyanide, Mathews found, were sufficient to retard or prevent the spontane-

ous oxidation of cysteine to cystine, both in neutral and alkaline solution. Mandelic nitrile was also found to check this oxidation, but capro-nitrile was without action. It was thought probable that these toxins act "by uniting with the sulphur group of the cysteine in the position that the oxygen ordinarily unites and thus check the oxidation. Possibly, the cyanides unite in the cell also with the labile sulphur atom of the albumins, since they escape from the mammalian organism as sulphocyanides."

The fact that the spontaneous oxidation of cysteine bears a certain degree of resemblance to the oxidations in living matter led Mathews to study the action of iron salts as well as the action of other metallic salts on cysteine oxidation. His experiments carried on quantitatively showed that an amount of iron a little more than $\frac{6}{1,000,000}$ M in concentration doubles the speed of oxidation of cysteine. In this oxidation the iron is supposed to act as an oxygen carrier, as it is presumed to do in the living cell; the oxygen of the air oxidizes the iron to a ferric salt which combines with the cysteine to form a violet-colored compound, this intermediate compound at once breaking up, "the iron passing a positive charge of electricity to the cysteine and becoming ferrous iron again. It then is reoxidized and the process is repeated."

Gold, platinum, mercury and copper salts also accelerate the cysteine oxidation in a neutral solution but not in an acid solution, but they are less powerful accelerators than iron salts. On the other hand, lead, nickel, cobalt, uranium, zinc and cadmium all inhibit the oxidation; they also retard or inhibit the accelerating action of iron.

Mathews believed that the behavior of these metals on the spontaneous oxidation of cysteine is related to their surface tension.

Cystine, when pure, was found to oxidize spontaneously in the air in an alkaline medium, but much slower than cysteine in neutral solution; during the process some sulfide and some ammonia were split off, but the products of the oxidation were not determined. Somewhat remarkable was the marked acceleration of oxidation by the combined action of potassium cyanide and ferric chloride in 0.5 N potassium hydroxide. The addition of potassium cyanide alone, of potassium ferrocyanide alone, or the iron salt alone had no effect, but the addition of the cyanide and iron salts together increased the speed of oxidation 100 per cent, or more. Finally, it should be added that Mathews' book *Physiological Chemistry*, published in 1915, is a comprehensive and valuable handbook of the subject, of special value because of the critical judgment displayed.

Among other workers at the University of Chicago who have contributed to the development of physiological chemistry the following must be referred to:

Fred C. Koch, associate professor 1919-1923 and professor of physiological chemistry since the latter date. A graduate of the University of Illinois, B.S., 1899, Ph.D., Chicago, 1912, he has been connected with the department of physiological chemistry at Chicago since 1909, his special interests being the study of phospholipins, secretins, and blood chemistry.

Martin E. Hanke, a graduate of the University of Chicago, B.S., 1918, Ph.D., 1921, who has been assistant

professor of physiological chemistry since 1925, and active in the investigation of the hydrochloric acid mechanism of gastric digestion and in the study of oxidases.

A. Baird Hastings, professor of physiological chemistry since 1926, who had his training largely in the Rockefeller Institute for Medical Research, especially with Donald D. Van Slyke. A graduate of the University of Michigan, B.S., 1917, Ph.D., Columbia University, 1921, he was at the Rockefeller Institute from 1921 to 1926 occupied with studies of gas and electrolytic equilibria in blood and of acidosis.

Rollin T. Woodyatt, since 1911 assistant professor of internal medicine in the Rush Medical College and a member of the staff of the Sprague Memorial Institute, who has done work on intermediate carbohydrate metabolism which has led to results of conspicuous value.

Esmond R. Long, associate professor of pathology since 1923, a graduate of Chicago University, Ph.D., 1919, who has been especially active in chemical pathology and has accomplished much in the study of the chemistry of the tubercle bacillus.

Milton T. Hanke, research chemist, Sprague Memorial Institute since 1918, and Karl K. Koessler also of the Sprague Memorial Institute, whose interests have been divided between internal medicine and biochemistry, and who have accomplished much work of chemico-physiological significance; the most important perhaps being their joint studies on *Proteinogenous Amines* from the Sprague Memorial Institute and the Department of Pathology of the University of Chicago, embracing some fourteen papers during 1920-1921, published in the *Journal of Bio-*

*logical Chemistry.* Among these studies are to be noted such topics as *The Quantitative Colorometric Estimation of Histidine in Protein and Protein-containing Matter; Is Histamine a Normal Constituent of the Hypophysis Cerebri?; The Relation of Histamine to Peptone Shock; The Production of Histamine and Other Imidazoles from Histidine by the Action of Microorganisms; A Microchemical Colorometric Method for Estimating Tyrosine, Tyramine, and Other Phenols; On the Electrotonic Interpretation of Certain Biochemical Phenomena.*

Katherine Blunt, who has been connected with the University of Chicago since 1913 as assistant professor and associate professor and from 1925 as professor of food chemistry and home economics, has been a stimulating influence at that center. A graduate of Vassar 1898, Ph.D., Chicago, 1907, she has been active in the study of basal, acid and mineral metabolism.

Still another worker at the University of Chicago, who, however, would be classified as a pathologist rather than as a physiological chemist is H. Gideon Wells. A graduate of the Sheffield Scientific School at Yale, 1895, M.D., Rush Medical College, 1898, Ph.D., Chicago University, 1903, he has been connected with the department of pathology at Chicago as assistant professor 1909-1913 and as professor of pathology since 1913. He has likewise been the director of the Sprague Memorial Institute of Chicago since 1911. Early in his career he recognized the important part chemistry was destined to play in the solution of many problems in pathology and consequently he devoted much time in perfecting his knowledge of organic chemistry, studying for a while at Berlin with Emil

Fischer. As a result he has been an active worker in the fields of general and chemical pathology and both directly and indirectly has done much to solve various problems of chemico-physiological importance. His book, *Chemical Pathology,* 1914, has gained wide recognition.

Finally, reference must be made to another worker at Chicago, whose accomplishments are of a high order, *viz.*, Florence B. Seibert. A graduate of Goucher College, 1918, a student of physiological chemistry in the Sheffield Scientific School at Yale, where she obtained the Ph.D. degree in 1923, she became the following year instructor in pathology at the university and assistant in the Sprague Memorial Institute at Chicago. Her work, largely of a chemical nature, particularly that bearing on tuberculin, will be referred to in the following chapter.

The studies of Wells on the chemical aspects of immunity are especially worthy of note and must be given brief consideration. To the chemist the vague and complex terminology which has gradually developed in the study of immunology has been and still is an obstacle to a clear understanding of this new field of chemistry, if such it can be called. Anaphylaxis and the so-called antigens, however, are terms clearly understood and to the physiological chemist convey a distinct and definite meaning, although there may be lacking precise comprehension of the character of the chemical reactions involved in anaphylactic phenomena.

On the subject of anaphylaxis the following papers from the pathological laboratory of the University of Chicago and the Sprague Memorial Institute may be referred to: *Studies on the Chemistry of Anaphylaxis,* by H.

Gideon Wells, 1908-1909; *Experiments with Isolated Proteins, Especially Those of the Hen's Egg,* by H. Gideon Wells, 1911; *The Antigenic Properties of Proteoses,* by Emanuel B. Fink, 1919, published in the *Journal of Infectious Diseases,* to which must be added a series of papers by Wells and Thomas B. Osborne of the Connecticut Agricultural Experiment Station, previously referred to in Chapter IV.

Wells early in his work discarded the use of the heterogeneous mixtures so commonly employed in anaphylactic experimentation and employed the purest forms of proteins obtainable. Thus, using pure crystallized egg albumin Wells found that one-twentieth of a millionth of a gram of the protein was sufficient to sensitize a guinea pig, so that distinct and typical symptoms were produced by a second injection of the same protein, while one-millionth of a gram of the protein was adequate to sensitize fatally. This hypersensitiveness to foreign proteins is, as Wells has expressed it, "one of the most spectacular phenomena discovered in immunity." What sort of a reaction takes place in the animal body by which it can be so sensitized as to respond to such infinitesimal quantity of a protein substance?

To quote Wells again, "it is a startling fact that a guinea pig, which can tolerate many cubic centimeters of such a protein mixture as horse serum in a single dose, will be almost immediately killed by as little as 0.01 cc. of this same serum, provided a similar or even much smaller amount has been injected into it ten days or more previously. The character of the death with violent con-

vulsions, perhaps within a minute of the time the injection is made, makes this observation all the more dramatic."

That the reaction, both sensitization and intoxication, must be due to the protein molecule seems perfectly certain, since it is quite inconceivable that any admixture having sufficient potency could be present in the injected protein, where such small amounts are employed, as to produce the effect noted. Again, the experiment with egg albumin affords a good illustration of the well-known fact that both sensitization and intoxication may result from the action of one and the same protein molecule. In such case the question arises as to whether the whole protein molecule is active in accomplishing both sensitization and intoxication, or whether one part of the molecule is responsible for the sensitization while another part causes the intoxication.

Many attempts to answer this and similar questions have been made without any convincing result. If, as has been claimed by several workers in this field, the intoxication that follows the second injection of protein into animals that have been sensitized by a previous injection is due to a cleavage of the protein molecule with liberation of toxic groups, it is conceivable that the intoxicating effect may be the result of some action on the part of the aromatic radicals of the protein molecule. The work of Wells has brought to light many interesting facts bearing on this question, some quite new, some simply confirmatory of the work of others.

Thus gelatin, the poorest of all proteins in aromatic radicals, was found to be lacking in the ability to participate in the anaphylaxis reaction, either with itself or with

other proteins; animals sensitized to egg white did not show any symptoms when injected with a solution of gelatin and conversely gelatin did not sensitize to egg white. Again, gliadin with its low content of tyrosine and phenylalanine was found to be weak in either intoxicating or sensitizing properties, presumably the former. Pure zein, on the other hand, was actively and specifically toxic to guinea pigs sensitized with zein, although this protein is lacking in both tryptophane and lysine. Plainly, the aromatic radical tryptophane does not take any part in the intoxication produced by proteins, or at least is not an essential factor.

However important the individual amino-acids may be in contributing to the anaphylactic properties of proteins, it is evident that it is the protein molecule as a whole that is antigenic; when the protein molecule is broken down the fragments are without antigenic action. Thus as Wells has found, even such large molecules as the proteoses, peptones, and polypeptides are without the power to either sensitize or intoxicate guinea pigs whether used in conjunction with themselves or with undigested egg white. Likewise, the crystallizable amino-acids were similarly inactive. In other words, as soon as proteins are altered or decomposed to any extent beyond the coagulable form their anaphylactic properties disappear. Conversion of egg albumin into acid albumin, however, did not, according to Wells, destroy its power to sensitize guinea pigs to egg albumin and to intoxicate the animals that had been so sensitized; both of these properties were, however, somewhat impaired. On the other hand, digestion of egg albumin with pepsin-hydrochloric acid tended to destroy

both the sensitizing and intoxicating properties, but very slowly.

As Wells has expressed it, "we do not know to what the proteins owe their antigenic activity." Tryptic digestion appears to furnish evidence, however, that it is the *protein* nature of the substances that is concerned in anaphylaxis; as digestion of serum, for example, proceeds and the coagulable protein grows less and less in amount, both sensitizing and intoxicating action diminish correspondingly. Wells found it extremely difficult to digest serum completely free from coagulable protein with trypsin. Thus, in one experiment the enzymolysis was continued for sixteen months, yet at the end of that time traces of coagulable material were still present, and doses of the serum of 1 cc. and over were able to sensitize guinea pigs to the homologous protein, although not fatally.

Equally interesting is the fact that crystallized egg albumin does not entirely lose its sensitizing power when heated in aqueous solution to 100° C. for fifteen minutes, if large doses are employed. Heating to 90° C., however, nearly destroys its intoxicating effect. Wells was inclined to the belief that the effects of heat in interfering with the sensitizing and poisonous action of proteins are due not to any chemical changes produced but simply to alternation in the solubility of the protein.

The chemical specificity of antigens from the same source as distinguished by the anaphylaxis reaction was clearly demonstrated by Wells with isolated proteins from the hen's egg. Thus, he found that ovovitellin from the yolk of hen's eggs when tested by the anaphylaxis reaction was entirely distinct from crystallized egg albumin

*and from ovomucoid from* hen egg white, and likewise different from the vitellin prepared from the yolk of turtle's eggs. Again, it was found that ovomucoid from the hen's egg produced characteristic and specific anaphylaxis reactions even after protracted boiling and after repeated precipitation with alcohol. Hence it is plainly quite distinct from crystallized egg albumin, from purified globulin of egg white and from ovovitellin.

In egg white (hen) there are, as Wells states, at least four antigens—three in the coagulable protein and one non-coagulable—which can be distinguished by the anaphylaxis reaction and are therefore biologically distinct; good examples of chemical specificity independent of species specificity. It is also to be noted that these antigens distinguished by the anaphylaxis reaction apparently correspond to the proteins which have been distinguished by chemical methods. Similarly, Wells and Osborne in their study of the purified proteins from cow's milk found that the four chemically distinct substances, casein, lactalbumin, lactoglobulin and an alcohol-soluble protein, when subjected to the anaphylaxis test were immunologically distinct.

Among the proteins of vegetable origin, the so-called proteoses obtained by Osborne from different seeds and grains were found by Wells and Osborne to be distinguishable by their biological reactions as well as by their chemical behavior. They possessed strong anaphylactogenic properties, causing very severe anaphylactic intoxication when injected into sensitized guinea pigs, even in doses of 0.001-0.0005 gram, such doses proving fatal with some of them. Further, their activity was not destroyed

by heating at 100° C. for one-half hour. These so-called proteoses are clearly chemically and biologically distinct from the other vegetable proteins so thoroughly studied by Osborne, the anaphylactic intoxication they produce greatly exceeding that induced by the reserve proteins of seeds in general. As stated in an earlier chapter these so-called vegetable proteoses must be quite different from the proteoses formed in pepsin and trypsin proteolysis, since the latter are not possessed of any anaphylactogenic properties whatever.

The work of Wells and Osborne with isolated pure proteins has strengthened belief in the view "that the antigenic capacity of a protein depends on the entire large colloidal molecular structure, while its specificity seems to reside in certain of the radicals of the molecule." The older belief that the immunological specificity manifested by individual proteins must be due to differences, too slight to be detected by any known method, either chemical or physical, has given way to the conviction that "immunological differences between proteins are usually, and as far as now known always, associated with and presumably dependent upon chemical differences which can be detected by chemical or physical methods." To this view the work of Osborne and Wells has contributed much, but while admitting that the specificity of the anaphylaxis reaction is connected with the chemical structure of the protein molecule, there remains the question what groups or radicals are concerned in these extremely delicate immunological reactions, where traces of protein almost unbelievably small are able to produce such startling physiological results.

Wells and Osborne were led to the belief that there must be at least two different groups or factors determining specificity within a single protein molecule. However this may be, the physiological chemist clearly recognizes that immunological methods are a great aid, and sometimes indispensable, in preparing proteins in a state of purity and may be employed to acquire information concerning the chemical relationship of proteins from different sources. Finally, reference should be made to the book by Wells, *The Chemical Aspects of Immunity,* 1925, published in the Monograph Series of the American Chemical Society, in which is presented a complete digest of the fundamental principles of immunology, from a chemical viewpoint, with a review of the experimental work upon which the conclusions drawn are based.

The subject of anaphylaxis or protein sensitization has naturally occupied the attention of many workers in this country as elsewhere, especially with reference to the action of infective bacteria and vaccine therapy, with the result that a great mass of data has accumulated and many hypotheses and theories have been advanced in explanation of the observations made. While most of these have little or no precise chemical significance and cannot be considered here, the early work of Victor C. Vaughan and his collaborators in the hygienic laboratory of the University of Michigan demands attention.

Among the many published papers from his laboratory, the following may be noted: *A Contribution to Cell Chemistry,* 1905; *Proteid Susceptibility and Immunity,* 1907; *On the Influence of Egg Albumin and its Cleavage Products on Animals; Upon Hypersensitiveness and Immunity,*

with Sybil May Wheeler, 1907; *Experimentelle Immunität gegen Coli- und Typhusbazillen*, with Miss Wheeler, 1908; *Die Spaltungsprodukte des Tuberkelbacillus und deren Einfluss auf Thiere*, with Miss Wheeler, 1908; the last two papers being published in the *Chemisches Centralblatt*.

At one time it was assumed that the bacterial proteins had a relatively simple molecular structure, but Vaughan and his co-workers, as well as many others, have shown that bacteria consist largely of gluconucleoproteins as complex in their chemical structure as the proteins of the higher plants and animals. Vaughan observed that when bacterial proteins as well as proteins of animal and vegetable origin, including egg albumin, were heated with twenty-five times their weight of sodium alcoholate (2 per cent sodium hydrate in absolute alcohol) for one or two hours at 78° C. they were split into two main fractions, one toxic, the other non-poisonous. The poisonous portion, similar but not identical in the different proteins, gave the ordinary protein reactions excepting Molisch, but was free from carbohydrate groups and contained no phosphorus, thus indicating that it was not a nuclein. This free poison he found killed guinea pigs in a few minutes when introduced subcutaneously or intravenously.

The symptoms induced in sensitized guinea pigs by the second dose of an unbroken protein were identical with those produced by a first dose of the free poison. Vaughan, from his many experiments, was led to the belief that the sensitized animal splits up the protein to which it has been sensitized with liberation of the poison contained in the molecule. In other words, in anaphylaxis there is de-

veloped in the body the capability of disrupting the specific foreign protein. From these and other observations Vaughan drew the conclusion that "every protein molecule contains a chemical nucleus, keystone or archon. This is the protein poison and is physiologically much the same in all proteins. One protein differs from another in its secondary or tertiary groups. In these resides the biological specificity of proteins. Biologically related proteins contain chemically related groups, and in these are found the sensitizing agents."

Vaughan considered it was clearly demonstrated that the sensitizing and toxic groups in the protein molecules are not the same. His theory of protein sensitization rests upon the view that every true protein will undergo disruption into a poisonous and a non-poisonous portion. To be sure, the exact structure and chemical nature of the poisonous groups could not be determined. The same was true of the sensitizing groups. The protein poison was plainly not an amino-acid, although it might be closely related to one of these. Vaughan indeed suggested that it might prove to be identical with histamine. He deemed it probable, however, that "the protein molecule contains a whole spectrum of poisons, one differing from another in some slight alteration in structure."

# CHAPTER VIII

Chemical study of the tubercle bacillus, work of Esmond R. Long, Hans Zinsser, Florence B. Seibert, Treat B. Johnson and Alice G. Renfrew, R. D. Coghill, Treat B. Johnson and Rudolph J. Anderson—Studies of the carbon dioxide absorption curve of human blood, acid-base equilibrium and other studies of blood, by John P. Peters and collaborators—Mayo Foundation, work of Walter M. Boothby and collaborators, Edward C. Kendall, oxidation-reduction systems—William M. Clark at The Johns Hopkins University—Mayo Foundation, work of Kendall and Ort, Kendall and Nord, reversible oxidation-reduction system, reduced and oxidized glutathione, Frank C. Mann and Carl H. Greene.

An English writer (F. G. Donnan) stated a few years ago that "looking into the future of chemistry he could see only two paths which were likely to lead to the opening up of vast fields of knowledge. They appear at first widely divergent, but some day they will converge and with startling results. The first is the investigation of the structure of the atom and molecule, and the second is the study of the chemical processes of the living cell."

To the student of biological chemistry, the latter part of this prediction is merely the expression of an opinion which has long been held, but the physiological chemist recognizes perhaps more fully than others the manifold difficulties which must necessarily attend the study of the chemical structure of living matter, and still more the chemical processes of the living cell. It is true, however,

that the great advances in organic and physical chemistry that have occurred during the past thirty years have opened up new avenues of approach which in the earlier years were quite inaccessible.

As an organic chemist (T. B. Johnson) has written recently, "It was as late as 1885 that the physiologist looked upon probably 90 per cent of the organic materials of cells or living tissue as organic constructions of unknown composition and structure. Preceding that time most of the fundamental organic changes occurring during life were enshrouded in mystery. Today the complex organic combinations of carbon, such as the fats, sugars, proteins, tannins, alkaloidal principles, terpenes, animal and plant pigments, purines and pyrimidines have been revealed in their true nature by the researches of organic chemists, and now those complicated constructions, the starches and celluloses, are also beginning to yield their secrets."

While it is true that much has been gained, that the chemical composition, constitution and genetic relationships of a large number of the compounds present in animal tissues and fluids are clearly understood, with consequent understanding of many of the phenomena of the animal body, yet it is equally true that the chemical reactions within the *living* cell stand somewhat apart. How far the characteristic components of living cells differ from the substances extracted from cells killed by the very first step of the analytical process employed we do not know. The molecular structure inside the cell wall during life may differ very materially from that which exists after the death of the cell. The metabolic activity of a single cell, such as a bacterium for example, is obviously

complex in the highest degree. In a cellular organism the physiological chemist is dealing "with a complicated organization of colloidal systems, while every organic constituent of the cell operates or functions in all its reactions under the influence of colloids."

In the existing state of knowledge the most that the physiological chemist can hope for is to ascertain as fully as possible the composition and constitution of the organic substances that enter into the structure of the dead cell, hoping thereby to draw inferences at least regarding the chemical processes that take place during life. Obviously, any cells that can be isolated in sufficient quantity to provide adequate material for study will yield results of great physiological importance, it being understood that each type of cell has its own peculiar composition determining its particular functions or lines of activity. In other words, the groups or types of cells, representing different microorganisms or different tissues of higher organisms, vary chemically and nutritionally.

In this country during recent years, there has been a vigorous movement inaugurated by The National Tuberculosis Association, to gain added light regarding the chemical composition of the tubercle bacillus; part of a general movement "to secure more accurate knowledge on the question of tuberculosis, which is undoubtedly the biggest single economic factor with which the country has to deal from the standpoint of health." In spite of the fact that study of the tubercle bacillus has been cultivated for many years in many countries with considerable progress in various directions, yet there remains profound ignorance of the *exact* chemical structure of the dead and liv-

ing cells. This is plainly a fundamental question, for there can be no hope of unravelling the complexities of cell activity without definite knowledge of the chemical structure of the cell components.

Today, there is expectation of a great forward movement in this direction, since increasing knowledge made possible through improved methods of chemical research promises substantial aid. Further, there is hope in the general plan of procedure adopted by The National Tuberculosis Association; a plan of coöperative research in which the best minds of the country are drawn upon for consultation and advice, while active workers [1] in various laboratories, representing different fields, are selected on the basis of knowledge and experience for individual lines of research.

Since we are dealing here solely with advance of knowledge in biological chemistry, it must suffice to point out some of the accomplishments in the chemical study of the tubercle bacilli during recent years by American workers.[2]

As is well known, the tubercle bacilli are able to grow on a chemically simple soil, a matter of considerable importance when the problem of securing a large crop of bacilli free from extraneous substances is considered. Esmond R. Long, of the University of Chicago, referred

[1] A group of some forty scientists who are working under the auspices of The National Tuberculosis Association in universities and laboratories all over the country.

[2] In this résumé, the author has drawn freely from the material contained in published reports in *The American Review of Tuberculosis*, *Transactions of The National Tuberculosis Association*, the *Journal of Biological Chemistry* and the *Journal of the American Chemical Society*, of the past three or four years.

to in the preceding chapter, has made a careful study of the metabolism of the tubercle bacilli with special reference to the materials needed for growth and the substances produced during growth. In harmony with knowledge gained by earlier workers he has found that the only compounds absolutely required for growth of the bacillus are glycerol, phosphoric acid, an amino group or ammonia, and a few inorganic salts, the glycerol being apparently the chief nutritional requirement.

Further, Long in emphasizing the well-established fact that the tubercle bacillus is characterized by a high content of wax has called attention to the fact that among the large group of acid-fast bacilli those which have a higher content of wax apparently require larger amounts of glycerol in the culture medium. In this connection he has also pointed out that those microörganisms that are least glycerophilic and low in their content of wax are least pathogenic. From this it might perhaps be inferred that the cell wax has its origin in part in the glycerol, and further that between the wax and the pathogenicity there is some causal relationship. The acid-fastness of the tubercle bacillus, Long considered to be due to a rather unstable protein-lipoid combination.

As to the substances produced by the bacilli and excreted from the cells, Long observed in harmony with the findings of earlier workers that the nutrient mixture in which the cells were growing tended to vary in reaction from acid to alkaline or the reverse, implying either a production of both acids and alkalies, or a withdrawal by the cells of one or the other, thereby producing a change in the reaction of the medium. Since amino-acids contain

both acid and basic elements, it is conceivable, as Long suggested, that the cells might use one of these elements more freely than the other, thus leading to a change of reaction.

Again, acid products might be formed in the metabolism of the glycerol, while the neutral phosphate salts used in the culture medium would naturally yield alkali as the phosphoric acid was taken up by the growing cells. Apparently, the only definite conclusion to be drawn was that both acid and alkali resulted from the growth of the bacilli and that they might originate in one or all the ways indicated. Using the amino-acids alanine and imidazolalanine or histidine in the culture media, Long endeavored to isolate the acid decomposition products, but was unable to detect either lactic or pyruvic acid in the alanine medium, and could find no evidence that the amines of the above amino-acids were produced.

Naturally from the viewpoint of the bacteriologist the most important product from the tubercle cell is tuberculin, but the chemical make-up of the active principle is quite unknown. Long, employing a culture medium free from protein, found that protein appeared in the medium in chemically detectable amount. As Long expressed it, "such media are active tuberculins, but this does not necessarily mean that the protein which has appeared is actually the active principle of tuberculin."

Hans Zinsser, professor of bacteriology and immunity at the Harvard Medical School since 1923, obtained tuberculin from the dried and ground tubercle cells by extraction with weak alkali. Treatment of this extract with acid caused a heavy precipitate, which was termed

phosphoprotein and nucleoprotein, although it was recognized that other substances were likewise present. The filtrate was heated to boiling at a pH of 5, the coagulated proteins removed, the filtrate on cooling sometimes yielding a little of Bence-Jones protein. Addition of alcohol to the final filtrate caused a precipitate which was active as tuberculin. This substance, as well as a corresponding substance obtained by similar methods from other microorganisms, Zinsser found gave no biuret reaction, no sulfosalicylic acid reaction, no Hopkins-Cole reaction, no ordinary protein reaction except a faint xanthoproteic. Obviously, these facts would tend to suggest that the active principle of tuberculin is not a protein.

In 1928, there appeared in the *American Review of Tuberculosis* two papers by Florence B. Seibert under the general heading *The Chemical Composition of the Active Principle of Tuberculin,* the one dealing with *The Fractional Heat Coagulation of the Protein of Tuberculin,* the other with *The Isolation in Crystalline Form and Identification of the Active Principle of Tuberculin,* these being the ninth and tenth papers in this series of studies. A water-soluble protein fraction of tuberculin was prepared by saturating raw tuberculin with ammonium sulfate and dialyzing the precipitate dissolved in water until free from the ammonium salt. On evaporation of the solution *in vacuo* below 50° C. to dryness all the specifically active material of the tuberculin was obtained.

Fractionation by heat might well be expected to throw some light on the nature of the active substance. The results of the various trials showed that "fractions coagulated at 50° C., 70° C., 80°-90° C., 97°-99° C. (boiling),

120° C. (autoclaving), and even the final non-coagulable fraction, which also definitely contained some whole protein, all gave equally marked tuberculin skin reactions, indicating that the specific biological activity is not due to any particular fraction but rather accompanies all fractions where the tuberculin protein is present." The author concluded that all fractions are probably derivatives of the same protein in varying stages of chemical change and are not necessarily different proteins.

Recognizing that the water-soluble protein of tuberculin, which is responsible for the specific skin reaction in tuberculous subjects, resembled in many of its properties ovalbumin, the attempt was made to crystallize the protein, using large quantities of tuberculin in one case 288 liters made on Long's protein-free synthetic medium and following Hopkins' method for crystallizing egg albumin. In this the author was eventually successful, thereby marking a very distinct step forward in that the isolation from tuberculin of a specifically active protein in such a state of chemical purity constitutes the first time that a crystalline protein of bacterial origin has been obtained. The crystals were in the form of burrs or bundles of fine needles generally mixed with amorphous particles. The optimum crystallization point was found to be pH 4.9, the possible range of pH being extremely narrow. "Even after fourteen crystallizations the protein gave maximum tuberculin skin reactions and caused a typical tuberculin atrophy in the testicle of a tuberculous guinea pig. A test made after ten crystallizations showed that less than half as much of the purified protein was required to produce an equally strong reaction as of the original fractions."

Especially noteworthy, however, is the fact that this water-soluble, crystalline protein which resembles an albumin, containing the ordinary amino groups but free from carbohydrate, is very unstable, readily undergoing "denaturization." When the latter takes place the protein will no longer crystallize and there is a change in solubility and a loss in biological activity. Finally, it should be added that up to the present it has not been found possible to prepare the crystals wholly free from amorphous particles; a fact assumed to be due to the great unstability of the protein, but which may possibly have other significance.

A great impetus to the chemical study of the tubercle bacillus has come through the possibility of growing large crops of the bacillus on a definite synthetic medium, such as Long's, by which admixture with the many complex substances, protein and others, present in the old-time culture media is done away with. A simple medium of known composition makes possible a fairly accurate study of at least some of the chemical changes taking place during the growth of the cells. Further, the facilities provided by commercial organizations for the production of bacteria on a large scale have rendered it possible to undertake systematic chemical studies with quantities of material hitherto unobtainable, and much work is being carried on at present in various directions. Treat B. Johnson, professor of organic chemistry at Yale, working with several collaborators in coöperation with The National Tuberculosis Association, has reported various findings, some of which may be referred to here.

With Alice G. Renfrew, a research fellow of The Na-

tional Tuberculosis Association, 1927-1928, experiments on changes in the culture medium during the growth of the bacilli were instituted with results as follows. Portions of Long's synthetic medium, two hundred cubic centimeters each, contained in suitable culture bottles were inoculated with human tubercle bacilli and incubated at 37° C. under sterile conditions. Each successive week for a period of two months samples of the growing cultures were removed for analysis, two culture bottles, however, being left undisturbed for a period of three months and two for a period of four months. The growth of the bacilli on this synthetic medium reached its maximum during the sixth week of incubation. During the first three weeks of incubation there was an increasing alkalinity, paralleled by an increase in ammonia, probably derived from the amide nitrogen of asparagine.

In harmony with the findings of other investigators, luxuriant growth was attended by a rapid production of acidity, the increasing acidity being accentuated by a decrease in the amount of ammonia in inorganic combination. It was also observed that the decrease in available phosphoric acid was not at all equivalent to the decrease in ammonia. At the end of four weeks' growth, the sudden change in pH was attended by the appearance of reducing substances (sugars) and of coagulable protein in the medium. "The cultures after the fifth week showed nearly five times as much coagulation at 60°C. as that observed for the fourth week, despite the small change in pH from 5.6 to 5.4." Very noticeable was the fact that during the later stages of incubation the rate of cell autolysis exceeded the rate of growth. "By the sixteenth week

autolysis had effectively cut down the yield of dry bacilli, having decreased the production by 30 per cent as compared with the average yield obtained during the second month."

The difficulties confronting the chemist in an attempt to isolate from the tubercle bacillus the various organic compounds present in the cell has been well expressed by Johnson, "Complete synthesis of highly developed organic structures, such as proteins and aromatic combinations, are brought about within a few hours as the new bacterial cells develop. Therefore, one might expect to find evidence of every intermediate stage of organic development at all periods of cell-growth from the time of generation to its death. In other words, our chemical problem of bacterial analysis is complicated and rendered very difficult by the fact that a bacterium is always operating as a completely organized chemical system, undergoing constantly fundamental chemical changes and manipulating at a very high reaction velocity, and with an accuracy and completeness that seem almost incomprehensible."

Work by R. D. Coghill in the Yale laboratory on *The Albumin-globulin Fraction of the Tubercle Bacillus*, 1926, and on *The Alkali-soluble Protein of Tubercle Bacillus*, 1926, published in the *Journal of Biological Chemistry*, has shown that the dry, defatted bacilli contain a water-soluble protein having the properties of an albumin and characterized by an unusually high content of basic amino-acids, arginine in particular being much larger in amount than is usually found in proteins, excepting the protamines. The globulin content of the tubercle bacillus, on

the other hand, was found to be very small, if indeed any was present. The albumin had marked biological activity. After removal of the water-soluble constituents from the tubercle bacillus, extraction of the cells with 0.5 per cent solution of sodium hydroxide yielded a protein containing 14.2 per cent of nitrogen and in amount equal to 20 per cent of the weight of the dry bacterial cell. This protein was radically different from the water-soluble protein, being practically free from tuberculin action, and containing much less basic nitrogen.

It is to be noted that of the total protein in the tubercle bacillus the water-soluble fraction represented only a small proportion—1.5 to 2 per cent—while the alkali-soluble fraction represented one of the largest units separated by analysis, and yet was not apparently possessed of any noticeable physiological action. Further, evidence was obtained that considerable protein exists in the cell in conjugated linkage, but as yet no insight has been gained as to the chemical nature of the combination.

Especially interesting is the presence in the tubercle bacillus of a large proportion of carbohydrate combinations, Johnson and R. J. Anderson, from the analytical data obtained, estimating that 10-12 per cent of the normal cell is made up of such combinations. Thus, polysaccharide configurations were found in the culture media in the alcohol-ether extract of the cells, and in various fractions of the defatted cell. Apparently both hexoses and pentoses take part in the growth of the tubercle bacilli As Johnson and Anderson have stated, "it seems that the growth cycle of the human cell is dependent on special autolytic changes leading to the production of sugar com

binations which approach maximum at time of maximum growth of the cell."

The lipoid material of the tubercle cell, as has long been known, constitutes a large fraction of the dry bacilli, 20-40 per cent, and is of great interest because of the peculiar character of the wax, phosphatides and fatty acids that compose it. Two papers from the Yale laboratory by Rudolph J. Anderson bearing on this subject may be referred to, *The Separation of Lipoid Fractions from Tubercle Bacilli*, 1927; *A Study of the Phosphatide Fraction of Tubercle Bacilli*, 1927, published in the *Journal of Biological Chemistry*.

The lipoids of the tubercle bacillus have been considered by many investigators as possessing important biological qualities, notably the power of stimulating abnormal cell development. Further, to certain of the alcohol-soluble lipoids specific antigenic properties have been ascribed. As a result of the large amount of work done by various investigators in the past, it has been generally understood that these lipoids are composed largely of wax and glycerides, with some phosphatides. Definite identification of many of these compounds, however, could hardly be expected in view of the small quantities of bacteria they had to work with.

The experimental work of Anderson on the other hand, as a part of the comprehensive coöperative research on tuberculosis now being conducted by American investigators, has special value in that he had at his disposal pounds of moist, living bacilli grown under definite conditions, on a simple medium free from proteins and other disturbing elements, and consequently he has been able

to isolate the various components in amounts that should make it possible to acquire definite knowledge of the exact chemical nature of these various lipoid substances.

Recognizing the importance of preserving the lipoid material in a condition as similar as possible to that in which it functions in the living cell, special precautions were taken to avoid alteration by heat or oxidation. The moist, living bacteria (grown on Long's synthetic medium for a period of six weeks, the human type of tubercle bacilli being employed, 2,200 cultures) were treated at room temperature with a mixture of alcohol and ether, followed by further extraction with chloroform, an atmosphere of carbon dioxide being maintained during all the operations. Further, the purified solvents used were saturated with carbon dioxide and the air was displaced from all vessels by the same gas before being used.

Anderson found the alcohol-ether extract contained glycerides and phosphatides together with a small amount of wax, the three groups being separated by the use of acetone. The alcohol-ether extract likewise contained some basic compounds, precipitable by mercuric chloride and by phosphotungstic acid, and considerable polysaccharide. The latter, when purified, Anderson found to be quite insoluble in alcohol and ether, and he attributed its presence in the alcohol-ether mixture to the water introduced by the moist bacteria. The chloroform extract yielded a large amount of crude wax, which when dried weighed 427 grams. The ether-soluble lipoids, as indicated above, were separated into an acetone-soluble fat fraction and an insoluble fraction composed of crude phosphatide which in turn was separated into two fractions. The phos-

phatide fractions were reported as possessing "unusually important biological properties," which are being investigated in the laboratories of the Rockefeller Institute for Medical Research and Cornell University Medical College.

Following is the tabular statement made by Anderson covering the "lipoid fractions and other compounds separated from 2,000 cultures of tubercle bacilli," from which it will be seen that the total lipoid material amounted to 23.78 per cent of the dry bacilli.

| *Fraction* | *Weight in Grams* |
|---|---|
| First phosphatide | 148.5 |
| Second phosphatide | 104.6 |
| Acetone-soluble fat | 240.0 |
| Chloroform-soluble wax | 427.0 |
| Base precipitated by $HgCl_2$ | 7.2 |
| Base precipitated by phosphotungstic acid | 5.3 |
| Polysaccharide | 33.9 |
| Dried bacterial residue | 2902.0 |
| Total weight | 3868.5 |

If any excuse is needed for this somewhat lengthy and detailed presentation, it is to be found in the illustration it affords of the advantages to be gained by a coöperative method of research, where facilities of all kinds can be had in large measure, and where the possibilities of gaining accurate knowledge are correspondingly increased.

In considering Anderson's work on the phosphatides of the tubercle bacillus it is to be remembered that at that date no *definite* knowledge existed regarding the nature and properties of these substances. He had in hand 194.5

grams of purified phosphatide, when freed from wax, equal to a little more than 5 per cent of the dry bacilli. Consequently he was able to hydrolyze relatively large quantities of the material with a view to identifying the component parts. As a result of his isolation of the different cleavage products, Anderson calculated the composition of the phosphatide to be per 100 parts

| | |
|---|---|
| Palmitic acid | 30.5 |
| Oleic acid after reduction to stearic acid | 12.8 |
| Liquid saturated fatty acid | 20.9 |
| Glucose | 13.9 |
| Sugar acid | 13.8 |
| Glycerophosphoric acid | 5.4 |

The nature of the liquid saturated fatty acid and the sugar acid are still to be determined. In this connection it is to be noted that Doctors Sabin and Doan, as a result of their biological studies with the phosphatide, found that "intraperitoneal injection of an aqueous suspension of the phosphatide exerted a marked stimulation on the monocytes and epithelioid cells leading to the formation of typical tubercular tissue." Further study has revealed that the liquid saturated fatty acid represents the biologically active principal of the phosphatide. Equally interesting is the fact that the so-called wax of the tubercle bacillus contains a similar, if not identical, fatty acid, which "possesses a specific stimulating effect on the proliferation of monocytes and epithelioid cells."

Finally, Johnson and Anderson have reported the results of the hydrolysis of the "purified wax" of the tubercle bacillus, and of the "soft wax" fraction, the latter

proving to be composed essentially of mixed glycerides. The cleavage products of the "purified wax" were made up of ether-soluble fatty acids corresponding to about 71 per cent, and water-soluble substances representing about 40 per cent of the original material. In the ether-soluble portion four different fatty acids were present, three being peculiar saturated acids not yet identified, while the fourth was an unsaturated acid, probably oleic. Of the saturated acids, the one already referred to as having marked biological properties appeared the most important. It melted at 18.5° C. and in alcoholic solution had a specific rotation of −1.6°. Preliminary analyses indicated that its formula was probably $C_{22}H_{44}O_2$.

The water-soluble constituents were composed largely of reducing sugars yielding osazones having the properties of pentosazones, and considerable glycerophosphoric acid with some free glycerol and free phosphoric acid. From these and other observations Johnson and Anderson concluded that the wax of the tubercle bacillus is "not a wax but a complex phosphatide containing a large percentage of a sugar complex."

Progress of this coöperative research, in which chemists, biologists and bacteriologists are actively engaged, may confidently be expected ultimately to yield results of great chemical and biological importance.

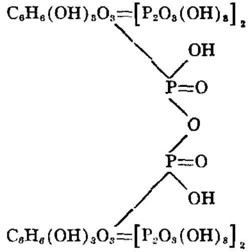
Rudolph J. Anderson, professor of chemistry in Yale University since 1927, was for many years biochemist at the New York Experiment Station at Geneva, 1911-1926. A graduate of Tulane University, B.S., 1906, Ph.D., Cornell University, 1919, he was broadly trained in chemistry at Upsala, Berlin, under Emil Fischer, and in London at

the Ludwig Mond Biochemical Research Laboratory of University College. His research work has been especially in the field of plant chemistry, his studies of the organic phosphorus compounds of plants being particularly noteworthy.

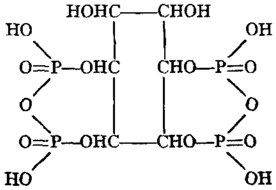
Among his many papers on phytin and phytic acid the following may be referred to: *Phytin and Pyrophosphoric Acid Esters of Inosite*, 1912; *Concerning the Organic Phosphoric Acid Compound of Wheat Bran*, 1914; *Concerning Phytin in Oats*, 1914; *Inosite Monophosphate, a New Organic Phosphoric Acid Occurring in Wheat Bran*, 1915; *The Hydrolysis of Phytin by the Enzyme Phytase Contained in Wheat Bran*, 1915; *The Utilization of Inosite in the Animal Organism*, with A. W. Bosworth, 1916; *Synthesis of Phytic Acid*, 1920; *Occurrence of Inosite Hexaphosphoric Acid in the Seed of the Silver Maple (Acer saccharinum)*, 1920; *Composition of Inosite Phosphoric Acid of Plants*, 1920.

The phytin of seeds and cereals has long been the subject of study, especially by Jordan of Geneva, the peculiar combination of inosite with phosphoric acid rendering it of special physiological interest. Anderson, as a result of his many investigations, came to the conclusion that the phytic acid of plants is inosite hexaphosphoric acid, $C_6H_6O_6(PO(OH)_2)_6$. Working with the organic phosphorus compound of wheat bran he found that the carefully purified and recrystallized barium and silver salts obtained therefrom showed on analysis close agreement with the calculated composition of the corresponding salts of the hexaphosphoric acid compound. By acting on dry inosite with dry pyrophosphoric acid at 200°-220°C.,

Anderson obtained a new pyrophosphoric ester, *viz.*, di-inosite tri-pyrophosphoric acid ester, corresponding to



Again, by a reaction between inosite and a mixture of phosphoric acid and phosphorus pentoxide, following the method of Posternak, Anderson obtained another inosite phosphoric acid which, while not identical in composition with the natural phytic acid and differing somewhat in its reactions and properties, contained nearly the same percentage of phosphorus. In composition this acid corresponded to inosite dipyrophosphoric acid, in which two hydroxyls of each molecule of the pyrophosphoric acid *had reacted with two alcoholic hydroxyls of the inosite*, as indicated by the following formula:



Anderson considered it possible, however, that the inosite might be combined with a more condensed phosphoric acid. Still another, previously unknown, organic phosphoric acid—inosite monophosphate—was isolated from wheat bran, the alkaline earth salts of which were not precipitable by ammonium hydroxide; differing in this respect from other known organic phosphoric acids as well as from ordinary phosphoric acid. A new inosite triphosphate was also obtained, thereby testifying to the presence of several inosite phosphate compounds in wheat, aside from phytic acid. Anderson's many efforts to synthesize phytic acid led him to the opinion that synthesis could never be effected by heating together inosite and phosphoric acid, for the simple reason that phytic acid is readily decomposed by heat into mixtures of inorganic phosphoric acid and lower inosite phosphoric compounds such as inosite tetraphosphoric acid.

The presence of an enzyme, phytase, in wheat and other cereals naturally suggested that hydrolysis of phytin might occur in the dried seeds and meal. Anderson found that the chief products formed from phytin by phytase in wheat bran were inorganic phosphoric acid and certain intermediate compounds corresponding to inosite tri-, di- and monophosphates. A portion of the phytin was completely hydrolyzed into phosphoric acid and inosite. The maximum activity of phytase was manifested in the presence of 0.1 per cent hydrochloric acid and 0.2 per cent acetic acid, while in the presence of 0.5 per cent hydrochloric acid there was no hydrolysis whatever.

By digesting wheat bran, corn meal, oats and cottonseed meal with 1.0 per cent hydrochloric acid, which destroys

the enzyme, Anderson separated from the extract salts identical with the tribarium phytate and heptabarium phytate; these seeds all contain the same organic phosphorus compound, *viz.*, phytic acid or inosite hexaphosphate. The seeds of the maple tree, Anderson found, also contain inosite hexaphosphate, but the powdered seed when allowed to dry for a year or two undergoes spontaneous hydrolysis into the pentaphosphoric acid compound.

The National Tuberculosis Association is only one of the many organizations that have turned to chemistry for aid in the solution of its problems. One of the first, if not the first, laboratories of medical research in this country was established in connection with the Bellevue Hospital Medical School in 1884 by the aid of Andrew Carnegie. Since that date, research laboratories connected with many hospitals throughout the country and independent laboratories or foundations, some of which have been mentioned previously, all having to do with increase of knowledge bearing on the causation of disease, have come into existence with results of inestimable value to medical science.

In all these institutions physiological or biological chemistry has occupied a conspicuous position, in harmony with the growing recognition of the importance of the chemical aspects of physiological and pathological processes. Further, it is worthy of note that the research work of these institutions has not been confined to narrow channels, with the sole object of practical application to some specific problem, but it has in many cases at least been broad in character with full recognition of the principle that

any increase in biological knowledge will ultimately prove of benefit to medical science and to humanity.

To workers in the field of internal medicine there are many avenues of research where chemical studies are of primary importance. Present-day knowledge of diabetes owes much to the purely experimental work carried on in laboratories of physiological chemistry, as well as to the more strictly clinical work conducted by the physician himself with the aid of his chemical training. Again, in cardiovascular diseases and in nephritis, where it is necessary to know something of the mechanism of the changes in blood and tissue hydration, extensive studies of the electrolytes of the blood are called for; studies, however, which can be carried on intelligently and successfully only by one thoroughly trained in the chemical methods of blood analysis.

From the Medical Service of the New Haven Hospital and the Department of Internal Medicine of Yale University there have come two important series of investigations by John P. Peters and several collaborators under the general titles, *Studies of the Carbon Dioxide Absorption Curve of Human Blood*, and *Total Acid-base Equilibrium of Plasma in Health and Disease*. A graduate of Yale, A.B., 1908, M.D. Columbia University, 1913, Peters gained broad experience in New York hospitals, and in research work at Columbia and Cornell Medical Colleges and as associate professor of medicine at Vanderbilt University, 1920-1921. At the Hospital of the Rockefeller Institute he collaborated with Donald D. Van Slyke and others in *Studies of Gas and Electrolyte Equilibria in Blood*, published in 1922. He came to Yale in 1921 as

associate professor of internal medicine, becoming professor of that branch of medicine in 1927.

In connection with his service at the New Haven Hospital Peters has accomplished so much in lines of research of a chemico-physiological character that a brief description of his work is called for here. The more important studies to which reference must be made are *The Relation of the Hemoglobin Content of Blood to the Form of the Carbon Dioxide Absorption Curve; The Construction of the $CO_2$ Absorption Curve from one Observed Point; The Relationship of the $CO_2$ of Blood to That of Plasma;* all three papers with Harold A. Bulger and Anna J. Eisenman, published in the *Journal of Biological Chemistry,* 1923.

The second group of papers include *The Concentration of Acids and Bases in Normal Plasma,* with Harold A. Bulger, Anna J. Eisenman and Carter Lee, 1925; *The Effect of $CO_2$ Tension on the Concentration of the Acids of the Plasma of Oxygenated Blood,* with Harold A. Bulger and Anna Eisenman; *The Differences Between Arterial and Venous Blood,* with Harold A. Bulger and Anna Eisenman, 1925; *The Effects of Stasis, Exercise, Hyperpnea, and Anoxemia; and the Cause of Tetany,* with Harold A. Bulger, Anna Eisenman and Carter Lee; *Miscellaneous Pathological Conditions,* with Harold A. Bulger, Anna Eisenman and Carter Lee, 1925; *A Study of Human Red Blood Cell Permeability,* with A. Maurice Wakeman and Anna Eisenman, 1927, all published in the *Journal of Biological Chemistry.*

From the many data presented in the above papers the following conclusions may be referred to as having

special chemico-physiological significance. In 1916 Hasselbach had shown by appropriate experiments that the hydrogen-ion concentration of blood could be calculated from the carbon dioxide tension and the bicarbonate concentration with a mean error no greater than that of the electrometric method, this in harmony with the deductions drawn theoretically by Lawrence J. Henderson eight years earlier. (See Chapter V.) Hasselbach transferred Henderson's equation

$$C_H = K \frac{[H_2CO_3]}{[B\ HCO_3]} \text{ into the logarithmic form}$$

$$pH = pK_1 + \log \frac{[B\ HCO_3]}{[H_2CO_3]}$$

By determinations of the $CO_2$ content of both blood and plasma together with the oxygen capacity and cell volume of blood exposed to known mixtures of air and $CO_2$, Peters and his associates obtained data which supported the contention of Warburg that $pK_1$ of the Henderson-Hasselbach equations shows an apparent variation with hemoglobin concentration and with pH. Based on quantitative estimations of $pK_1$, curves were prepared by means of which corrections could be made, thereby rendering it possible by the use of such curves to predict the pH of plasma from the $CO_2$ content and $CO_2$ tension of whole blood, with a mean error of less than $\pm$ 0.01 pH.

Studying the electrolyte equilibria in blood, data were secured permitting an evaluation of the variations of $pK_1$ in whole blood. "The purely logarithmic straight line relation was found to agree with observed data better than does the pH — [B $HCO_3$] relation. This was especially

true at low $CO_2$ tensions." The total electrolyte equilibrium of the plasma in health and disease was studied by determining at the same time the total base, the inorganic acids, bicarbonate, chloride, phosphate and protein, the difference between the total base and the sum of the base-combining powers of the acids enumerated giving a measure of the organic acid and sulfate. Normal serum was found to contain 147 to 161 millimols of monovalent base, 138 to 148 millimols of this base being combined with the four acids protein, bicarbonate, chloride and phosphate.

Peters considered it probable that these limits should be extended for hospital patients to 145-167 for total base and 135-155 for total acid. The organic acid, it was found, never exceeded 20 millimols in normal persons or patients with pathological conditions in which there was no reason to expect a disturbance of electrolyte equilibrium. Finally, there was observed a general tendency for protein, bicarbonate and chloride to reciprocate in their changes; thus aiding one another in maintaining the total acid and total base at a constant level.

In considering the effect of $CO_2$ tension on the concentration of the acids of the plasma in oxygenated blood, Peters and his associates found that the sum of the base-combining powers of the acids $H\bar{C}O_3$, $\bar{C}l$ and protein of the plasma increases on an average about 2 millimols, when the $CO_2$ tension of the blood is increased from 30 to 60 mm. at 38°C. In this change $H\bar{C}O_3$ increases 5 millimols, the extent of the increase being determined chiefly by the concentration of hemoglobin or the volume of the blood cells. As Peters stated, the cells swell slightly,

thus decreasing the volume of the plasma, thereby augmenting somewhat the concentration and base-combining power of the protein, and to a less degree of the phosphate. This, however, may be more than offset by a diminution of the acid value of the protein caused by reduction of pH. The average change in plasma volume was found to be —0.6 volume per cent, while the base-combining power of the proteins diminished about 0.8 millimols. $\bar{Cl}$ decreased by about 2 millimols, "thus compensating for a little less than one-half of the $H\bar{CO}_3$ change." Since the base does not traverse the cell membrane it follows that the "loss of water from plasma to cells results in a concentration of base that neutralizes the excess acid."

Contrary to the "general conception that arterial and venous blood differ as regards electrolyte equilibria only in so far as they contain more or less carbon dioxide and water," Peters has shown through determination of oxygen capacity, cell volume, plasma proteins and whole blood chlorides that arterial and venous blood may also differ in their content of water and chlorides, the carbon dioxide absorption curves of the two bloods showing differences in harmony therewith. It is for this reason, Peters believes, that attempts to obtain proper respiratory quotients by simple comparison of the differences in oxygen and carbon dioxide contents of arterial and venous blood have usually been unsuccessful. In the transformation from arterial to venous blood, the end-result on the plasma acids $H\bar{CO}_3$ and $\bar{Cl}$ is an average alteration of 2.5 millimols, usually an increase. "The maximum changes encountered in fourteen examinations of nine patients, se-

lected because it was expected they would present large *venous-arterial differences* were +5 and −2.5 millimols."

Manifold experiments and observations on the effects of stasis, exercise, hyperpnea, etc., on electrolyte equilibrium led to certain well-defined results, a few of which may be quoted here. Prolonged venous obstruction causes a "transfer of water from the blood to the tissues and a concentration of the proteins. Base combined with bicarbonate is unavailable for the neutralization of this excess acid because the usual escape of $CO_2$ through the lungs is prevented by the presence of the tourniquet. Under these circumstances plasma chloride diminishes, yielding its base to protein and carbonic acid."

"If overventilation is produced as rapidly as possible, symptoms of tetany appear when the pH has risen by not more than 0.2. Although the total $CO_2$ of the serum falls, the carbon dioxide capacity remains unaltered. Organic acid, probably partly ketone acids, but mostly lactic acid, is considerably increased. The total base remains unchanged and the base required for neutralization of the foreign acids is largely derived from the chlorides, which are diminished. The reaction of the electrolytes to oxygen-want varies according to the respiratory response. If moderate overventilation develops and continues for a long time the bicarbonate falls. The $HCO_3$ is replaced partly by an increase in the concentration of serum protein, but chiefly by Cl withdrawn from the tissues."

The whole tendency of the reactions taking place whenever there is a disturbance of electrolyte equilibrium is towards the "restoration of equilibrium and the mainte-

nance of the functional automatism of the whole organism." In other words, the effort made is not apparently to hold any single constituent or group of constituents at a given level of concentration, but rather to set in motion such a train of reactions involving all the electrolytes as will best restore equilibrium as a whole.

Obviously in the distribution of water and electrolytes between the serum and blood cells the permeability of the cell membrane must be taken into account. Employing more refined methods than were available at the time of Hamburger's oft-quoted experiments (1910-1916), Peters and his collaborators found "that when electrolyte equilibrium was disturbed by the addition of salt or water to blood even beyond the extreme limits of variation recorded in human blood, the red blood cell membrane apparently remained impermeable to the cations sodium and potassium." Hence, it would appear that the re-establishment of electrolyte equilibrium after disturbance caused by the addition of potassium or sodium chlorides or carbonates to blood must be accomplished by the transfer of water, carbon dioxide and chlorine across the cell membrane.

The Mayo Foundation for Medical Education and Research, established in 1915 by William James Mayo and his brother Charles Horace Mayo at Rochester, Minnesota, in affiliation with the University of Minnesota, the forerunner of which was the Mayo Clinic of St. Mary's Hospital, Rochester (1889), has long been recognized as one of the outstanding research centers in the country. Here, all phases of medical research have been carried on with results of the greatest value, biochemical studies having special prominence.

Among the workers along chemical lines, Walter M. Boothby, associate professor of medicine and head of the section of clinical metabolism, has accomplished results that demand attention. A graduate of Harvard University, A.B., 1901, M.D., 1906, he has been especially active in the study of metabolic problems, the papers to which reference will be made being *A Comparison of the DuBois and the Harris and Benedict Normal Standards for the Estimation of the Basal Metabolic Rate*, 1922; *Summary of the Basal Metabolism Data on 8614 Subjects with Special Reference to the Normal Standards for the Estimation of the Basal Metabolism Rate*, 1922; *A Quantitative Estimate of the Catalytic Power of Adrenalin and Thyroxin as Calorigenic Agents and the Relative Rate of Their Destruction*, 1923; all published with his co-worker Irene Sandiford in the *Journal of Biological Chemistry*.

Other studies dealing especially with pathological conditions, such as basal metabolism in acromegaly and nitrogen equilibrium in exophthalmic goiter are also worthy of note. Boothby's extensive studies of basal metabolism led him to the conclusion that the DuBois height-weight formula for the determination of the surface area and the DuBois normal standards for age and sex based on calories for each square meter of body surface constitute the best standards at present available for predicting the normal heat production.

In his study of the influence of thyroxine and adrenaline on heat production, Boothby found from experiments with a myxedematous patient that the injection of thyroxine was far more effective in increasing heat production than adrenaline; one milligram of adrenaline increasing the

basal metabolism only 50 calories, while a like amount of thyroxine caused an extra heat production of 1,008 calories. From his data he estimated that one molecule of thyroxine as a catalytic agent in the production of heat is 64 times more effective than one molecule of adrenaline.

Finally, reference must be made to a later piece of work by Boothby, entitled *A Study of the Nitrogen Minimum,* with Harry J. Deuel, Jr., Irene Sandiford and Kathleen Sandiford, 1928, in which they studied "the effect of sixty-three days of a protein-free diet on the nitrogen partition products in the urine and on the heat production." The experiment as a whole extended through eighty-one days, divided into periods, the subject being a normal healthy man. During the first period of 30 days, on a nitrogen-free diet rich in carbohydrates, the daily intake of the latter amounted to 370 grams for the first 7 days, followed by 440 grams per day for 23 days, in order to establish a constant base line and a minimal excretion of urinary nitrogen. The results of the experiment were summarized by Boothby as follows: The minimal excretion of total nitrogen through the urine during this first 30-day period amounted to 2.10 grams per 24 hours.

In a second period on a practically protein-free diet, after much greater exhaustion of the deposit protein following the administration of thyroxine, the nitrogen excretion fell to 1.75 grams on the last day of the period, equal to 0.0241 gram per kilogram of body weight. This amount, as Boothby states, is slightly lower than any on record. The total loss of deposit protein nitrogen through

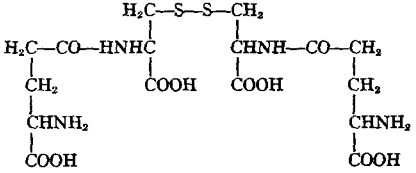
the urine during the experiment was 259 grams, while the loss of body weight was 11.3 kilograms. On the assumption that the total loss of nitrogen—through the urine and feces—would amount to 291 grams, it would mean a loss of 1.8 kilograms of dry protein, which with its normal complement of water would equal about 12 kilograms, or about 16 per cent, of the original body weight. It is significant that no noticeable deleterious effects followed this loss of tissue protein. Equally significant is the apparent fact that the quantity of reserve or deposit protein in the body must be much larger than has generally been assumed.

Boothby found that the variations in total urinary nitrogen were due almost entirely to variations in the amount of urea excreted, the output of creatinine, uric acid, neutral sulfur and ethereal sulfate remaining extremely constant. The same was true of the amino-acid and undetermined nitrogen. He concluded that the true endogenous metabolism of a normal adult man is slightly less than one gram daily. Regarding the basal metabolic rate a rapid fall was observed during the first eight days of the protein-free diet, coincident with the rapid decrease in the free utilization of deposit protein. Thereafter, when the nitrogen excretion dropped to lower levels, the basal metabolism remained essentially constant. When, however, the subject returned to a high protein diet with consequent replenishing of his stores of reserve or deposit protein, his basal metabolism rose to a level above the normal.

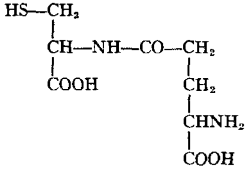
Another worker at Rochester is Edward C. Kendall, a graduate of Columbia University, B.S., 1908, Ph.D., 1910,

head of the section of chemistry of the Mayo Foundation and professor of biochemistry at the University of Minnesota, whose successful investigation of thyroxine has brought him well-deserved recognition. He has likewise done much work on the subject of oxidation in the animal organism, his researches on *The Oxidation-reduction Potentials of 2-Oxydihydroindole-3-proprionic Acid and Some of its Halogen Derivatives*, with John M. Ort, 1926, and on *Reversible Oxidation-reduction Systems of Cysteine-cystine and Reduced and Oxidized Glutathione*, with F. F. Nord, 1926, being especially noteworthy.

The discovery of glutathione as an auto-oxidizable component of animal cells in 1921 by Sir Frederick Gowland Hopkins of the School of Biochemistry, University of Cambridge, and the later work of Hopkins and Dixon, 1922, and of Dixon and Quastel, 1923, dealing with the function of "glutathione as a thermostable oxidation-reduction system" and with "cysteine and glutathione as a new type of reduction-oxidation system" have given an added stimulus to the study of the mechanics of cell oxidation. The chemical structure of oxidized and reduced glutathione as worked out by the English investigators is as follows:




*oxidized glutathione*



*reduced glutathione*

Hopkins and his collaborators considered reduced glutathione to be a dipeptide containing cysteine and glutamic acid, while oxidized glutathione was looked on as a combination of four amino-acids in which the groupings $\begin{matrix}-SH\\-SH\end{matrix}$ are changed to —S —S— as shown in the above formulas. According to this view when reduced glutathione undergoes oxidation the reaction would be $\begin{matrix}-SH\\-SH\end{matrix} + O_2 = -S-S- + H_2O_2$. Thus reduced glutathione is a hydrogen donator to molecular oxygen, which acts as hydrogen acceptor. The oxidized glutathione G — S— S— G can now again act as hydrogen acceptor and the process of alternate oxidation and reduction be continued; a reversible oxidation-reduction process of great significance. As to the mechanism of the oxidation and reduction in the living cell, it has been suggested that the activation of the molecule may be due "to the application of an external electric field." [8]

[8] Since the above statements regarding glutathione were written, there has appeared in the *Journal of Biological Chemistry,* October, 1929, an article by Sir Frederick Gowland Hopkins, under the title "On Glutathione; A Reinvestigation," in which certain errors in his previous work are corrected and the conclusion reached that glutathione is in reality a tripeptide, apparently

Kendall and Ort, working with the lactam 2-oxydihydroindole-3-propionic acid in connection with Kendall's study of the synthesis of thyroxine, found that atmospheric oxygen will remove two atoms of hydrogen from this indole derivative forming a bond from carbon 7 to the nitrogen 6, with production of 2, 3-imino-phenyl-*a*-glutaric acid; these two substances being the reduced and oxidized form of this compound. It was likewise found that this lactam will also reduce some dyes, such as dibromo- and other indophenols. The discovery of these facts led to a comprehensive study of the oxidation and reduction potentials of this series of compounds.

In their experimental work Kendall and his associates employed the methods so carefully worked out by William Mansfield Clark in his *Studies on Oxidation-reduction.* Clark, at present professor of physiological chemistry at The Johns Hopkins University, was from 1910 to 1920 chemist in the Bureau of Dairy Industry, United States Department of Agriculture, becoming in 1920 professor of chemistry in the Hygienic Laboratory of the United States Public Health Service where he accomplished a *large amount of valuable work on the determination of hydrogen ions,* and *reversible oxidation-reduction of organic compounds.* Especially important were the experiments carried on with the assistance of Barnett Cohen and H. D. Gibbs, bearing on the general principles underlying certain theories of oxidation and giving data on the

containing the three amino-acids, glycine, glutamic acid and cysteine. Another paper from the Cambridge laboratory in the same number of the *Journal of Biological Chemistry,* by Norman W. Pirie and Kathleen Godwin Pinhey, under the title "The Titration Curve of Glutathione," should be consulted.

equilibrium potentials obtained with mixtures of the oxidant and reductant of such compounds as *o*-cresol indo-2-6 dibromophenol and thymol indo-2-6 dibromophenol, published in the Public Health Reports, 1923 and 1924, of the United States Public Health Service. Especially valuable has been the book by Clark, the first edition of which appeared in 1920, under the title *The Determination of Hydrogen Ions; an Elementary Treatise on the Hydrogen Electrode, Indicator, and Supplementary Methods, with an Indexed Bibliography on Applications,* which has been widely used.

Kendall and Ort found that when a mixture of the reduced and oxidized forms of 2-oxydihydroindole-3-propionic acid was placed in a phosphate solution buffered to pH 7.4, it failed to produce a potential which could be related to the concentration of the oxidized and reduced forms by the usual equation. In fact, the potentials were characterized simply by fluctuations and drifts which could not be explained by changes in temperature, light or the presence of impurities. If, however, mild oxidizing agents were added to the solution, a gradual oxidation of the lactam occurred, resulting in an increase of the reducing potential to a point much higher than that given by the reduced form of the lactam alone.

By employing 2,6-dibromoindophenol as the oxidizing agent, these fluctuations and drifts in the potential were eliminated and solutions were produced which could be duplicated, with results of quantitative value. During the process of oxidation, there was no evidence of autocatalytic action, the oxidizing agent apparently reacting in stoichiometric proportion with the lactam. In this inter-

action between the lactam and dibromoindophenol the dye could be completely reduced and a reducing potential of +0.1 volts produced.

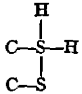
From their many experiments Kendall and Ort came to the conclusion that oxidation of this lactam with dibromoindophenol probably takes place in two stages; the first being the formation of an addition compound between the lactam and the oxidizing agent, presumably through the atom of nitrogen. In the second stage of the process they considered that this addition product undergoes a rearrangement with elimination of two atoms of hydrogen, resulting in a reduction of the dye and production of the oxidized form of the lactam, which has no effect on the platinum electrode. The active intermediate compound it was assumed must be the addition product between the dye and the amine group of the lactam.

Since the oxidized form of the lactam does not affect the platinum electrode the inference was drawn that between this oxydihydroindole and cysteine-cystine there is a certain degree of resemblance. Finally, although 2-oxy-2,3-dihydroindole-3-propionic acid and *a*-(2,3-iminophenyl) glutaric acid are the fully reduced and oxidized forms of this indole derivative, a mixture of the two compounds, Kendall and Ort found, does not form a reversible oxidation-reduction system, and they were unable to find any substance which would bring these two compounds into equilibrium with each other.

Passing now to glutathione and cysteine, the suggestion that these two compounds belong to a new system of substances, only one form of which (the reduced) affects the platinum electrode, together with the fact that the

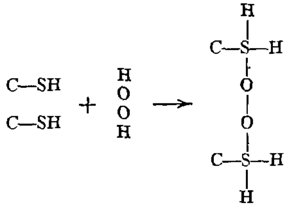
reducing potentials of cysteine and glutathione are independent of the presence or absence of their oxidized forms, led Kendall and his associate Nord, with the experiments on oxydihydroindole before them, to extend the observations and results of previous workers and to establish the conditions necessary and sufficient to form a reversible *oxidation-reduction* system with reduced and oxidized glutathione.

They confirmed the findings of the English observers, Dixon and Quastel, concerning the drift in the potential, but they ascribed the cause to changes occurring in the sulfydryl group rather than to the influence of the electrode. They considered that the probable change in the sulfydryl group responsible for the drift in the potential was a combination of two molecules of cysteine, with formation of a compound for which the following formula was suggested:

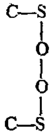


Kendall and his associate also found that in the presence of indigocarmine or other hydrogen acceptor, hydrogen dioxide and sodium disulfide will react with cysteine forming an addition product. In solutions containing cysteine and cystine, or GSH and GSS, with the oxygen or sulfur addition product, the potential was observed not to vary with the concentration of cysteine alone, but it was the ratio of cysteine to cystine that determined the absolute value of the oxidation-reduction potential at the

equilibrium point. In such a solution, reduced indigo was rapidly oxidized and indigocarmine was rapidly reduced. In the absence of the oxygen addition product, however, cysteine could not reduce indigocarmine, and cystine was unable to oxidize reduced indigo. Kendall considered that the only explanation of the reduction of the indigocarmine is in the interacting of the oxygen addition product with cysteine, thereby sensitizing the hydrogen of the sulfydryl group to a state of reactivity sufficient to bring about the reduction. The oxygen addition product must be a compound that can exist in a reduced and oxidized form and its possible structure in the case of cysteine, Kendall suggested, might be as follows, assuming that hydrogen dioxide combines with cysteine:

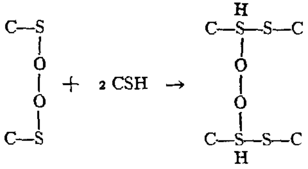


In the presence of mild oxidizing agents the four atoms of hydrogen could be removed and the molecule become



*oxidized form of the oxygen addition product.*

If two molecules of cysteine now combine with the oxidized form of the oxygen addition product the molecule becomes



However much uncertainty there may be regarding the soundness of the above suggestions, Kendall's results with indigocarmine clearly established the fact that the energy of the SH grouping can be made available for reduction, while the oxidation of reduced indigo demonstrated that the energy of the SS grouping can be made available for oxidation. Further, it was clearly shown that the same solution containing cysteine, cystine and the oxygen addition product will function either as a reducing or as an oxidizing agent, dependent solely on the addition to the solution of substances which change the potentials a little above or below that of the equilibrium point of the solution of cysteine-cystine. Cysteine and cystine alone, however, do not form a reversible oxidation-reduction system.

With reduced and oxidized glutathione, Kendall and Nord produced solutions having a fixed equilibrium point which would oxidize reduced indigo and reduce indigocarmine in a manner similar to the action of cysteine and cystine, the essential intermediate compound of this reversible system being an oxygen addition product of reduced glutathione. Additional results indicated that the

reduced and oxidized forms of glutathione are relatively stable substances in which the atom of sulfur is unable to change its state of oxidation with sufficient ease to influence physiological processes of oxidation and reduction. "Under certain narrow but definite conditions, glutathione can exist in the form of a highly reactive oxygen addition product in which the atom of sulfur changes its state of oxidation with every addition of suitable oxidizing and reducing substances in the solution. The more stable SH and SS forms of glutathione can react with the oxygen addition product, and the three forms of this compound make a reversible oxidation-reduction system."

Kendall's important work on thyroxine will be considered in a later chapter.

Reference must also be made to the accomplishments of Frank C. Mann, professor of experimental surgery and pathology, Mayo Foundation, and director of the division of experimental surgery and pathology of the Mayo Clinic, since he has been responsible for considerable work of a strictly chemico-physiological character. We can, however, refer here only to his *Studies on the Physiology of the Liver,* of which the three following may be specified: *The Effect of Insulin on the Blood Sugar Following Total and Partial Removal of the Liver,* with Thomas B. McGrath, 1923; *Muscle Glycogen Following Total Removal of the Liver,* with Jesse L. Bollman and Thomas B. McGrath, 1925; *The Specific Dynamic Action of Glycocoll and Alanine with Special Reference to the Dehepatized Animal,* with Charles M. Wilhelmj and Jesse L. Bollman, 1927; published in the *American Journal of Physiology.*

In the latter study it was found that when glycocoll and alanine were introduced intravenously into normal dogs, a definite specific dynamic action was produced with the usual change in the excretion of nitrogen through the urine, such as ordinarily follows the injection of amino-acids; but when the liver was removed, glycocoll and alanine failed to produce this effect.

In their study of glycogen, it was observed that in dehepatized dogs the glycogen of the muscles decreased proportionally to the decrease of blood sugar; that when glycogen was introduced intravenously it was converted into glucose and utilized by the hypoglycemic animal. "The symptoms which appear with a definite hypoglycemic level (0.035 to 0.025 per cent) bear no relation to the absolute amount of glycogen in the muscles and appear while there is still sufficient glycogen to bring the blood sugar to normal if it were released." In other words, the glycogen of the muscles cannot be drawn upon rapidly to sustain the normal level of sugar in the blood. Hence, it follows that the liver must be the main source of glucose in the blood and that the muscles of the body are of very little, if any, help in the maintenance of the normal content of blood sugar.

Still another piece of work from the Mayo Clinic and Mayo Foundation may be referred to, *viz., The Amino-Acid Content of the Blood in Normal and Pathologic Conditions,* by Carl H. Greene, Kathleen Sandiford and Helen Ross, 1923, in which it was found that the amount of amino nitrogen in the blood varies between 4.8 and 7.8 milligrams in 100 cc., the average being 6.3 milligrams.

These figures were obtained by the examination of normal plasma and by more than 400 observations covering 20 pathological conditions. It was also found that the level of amino nitrogen may be increased by flooding the organism with amino-acids arising during digestion or from rapid autolysis of body tissue, as in acute yellow atrophy of the liver.

# CHAPTER IX

Specialization in physiological chemistry—Willey G. Denis at Tulane University—Kastle and Loevenhart at State College of Kentucky—University of Louisville, Henry G. Barbour—University of Virginia, Alfred Chanutin—Vanderbilt University, Glenn E. Cullen—University of Texas, Byron M. Hendrix—University of Missouri, Charles W. Green, Robert B. Gibson, Albert G. Hogan —University of Iowa, Elbert W. Rockwood, Henry A. Mattill, Amy L. Daniels—Northwestern University, John H. Long, George L. Foster—University of Nebraska, Sergius Morgulis—Ohio State University, John F. Lyman—Merrill-Palmer School of Detroit, Icie G. Macy—Western Reserve University, John J. R. Macleod—University of California, T. Brailsford Robertson, Carl L. A. Schmidt, Edward S. Sundstroem, Herbert M. Evans, Martha R. Jones, Helen B. Thompson, Ruth E. Okey—Stanford University, Robert E. Swain—Food Research Institute, Carl S. Alsberg and Alonzo E. Taylor—University of Colorado, Robert C. Lewis—University of Indiana—University of Illinois, Harold H. Mitchell, Harry S. Grindley.

As has been indicated in previous chapters the multiplicity of laboratories for study and research in physiological chemistry constitutes one of the most convincing evidences of the great interest in and development of the science in this country. Further, the great diversity of topics under investigation, the wide range in the fields of study pursued, likewise testify to the striking growth of this branch of biology. The evolution of physiological chemistry, as in the development of most branches of science, has naturally been attended by more or less special-

ization, resulting in a tendency towards limitation of one person's activities to some particular branch or section of the science.

In the earlier years a worker might range from one field to another with reasonable success; he might indeed become proficient in the entire domain of physiological chemistry. Today, however, all this has changed with the rapid development of the past thirty years, and the individual worker must of necessity limit his activities to some special field in which he may hope eventually to become especially proficient and perhaps a recognized authority.

Again, the growth of knowledge in chemistry and physiology which has resulted in the more complicated machinery of modern methods and experimental procedure, has led to successive division, so that what was at one time a recognized field of research has become subdivided into sections, each one of which calls for special knowledge and for a master worker who can give all his energies to its cultivation and development. Thus in the study of metabolism, for example, the problems of respiratory exchange or gaseous metabolism may well occupy all the time and thought of one person, while the problems of protein metabolism and the intermediate changes connected therewith may demand all the time and special knowledge of another.

Further, in the study of the oxidation and reduction processes of the animal body still another line of inquiry is opened up which calls for a master worker versed in a different branch of chemical procedure, while the study of the acid-base equilibria of the body—blood, tissues and

excretions—demands training and experience which can come only from zealous efforts long continued. Truly, hyperspecialization in many directions is a necessary concomitant of progress today in physiological chemistry.

Again, physiological chemistry, or biochemistry, has found such wide application in the solving of problems in other branches of science as in agriculture, bacteriology and public health, and in certain branches of industry such as dairying, canning of food products, production and purification of corn products, sugars, fats and oils, that it is easy to realize something of the progress which has come from the solution of what may be termed borderline problems. While in the above lines of work benefits may be widely distributed, yet physiological chemistry itself has received much that has been helpful; many a fruitful idea or suggestion having come from the solution of problems which have no apparent biochemical significance.

In other words, the progress of physiological chemistry in this country during the past thirty years has come about largely through mass action, in which a large number of workers distributed over the entire land and occupied with widely divergent problems have contributed, some more and some less, to that fund of knowledge upon which the science rests. Look at any section of the country, whether it be the far south, the middle west, the eastern seaboard, or the Pacific coast, and in the universities, medical schools, hospitals, health centers, agricultural experiment stations, and special research institutions to be found there, workers in physiological chemistry will be plainly in evidence occupied with teach-

ing and research, all contributing something to the increase of biochemical knowledge.

At Tulane University, Willey G. Denis, a graduate of that institution in 1899, Ph.D. of Chicago University, 1907, a co-worker with Folin at Harvard and the Massachusetts General Hospital, 1913-1920, has been connected with the department of physiological chemistry at Tulane since 1920 as assistant and associate professor, and professor of biochemistry in the Medical School of that university from 1925 up to the time of her death in 1929. An exceedingly active worker, she elaborated with Folin a large number of methods for the determination of various organic and inorganic constituents of the blood and urine.

While at Tulane some of her more important pieces of work were the following: *Sulphates in Blood,* 1921; *On the Selective Action of the Kidneys as Regards the Excretion of Inorganic Salts,* 1922; *A Study of the Inorganic Constituents of the Blood Serum in Nephritis,* with S. Hobson, 1922; *A Study of the Inorganic Constituents of the Blood in Experimental Nephritis,* 1923; *A Study of the Effect of Temperature on Protein Intake,* with P. Borgstrom, 1924; *On the Distribution of Injected Sulphate in Tissues,* with Stella Lecke, 1925; *On the Distribution of the Non-protein Sulphur of the Blood between Serum and Corpuscles,* with Lucile Reed, 1927; *Concerning the Effect Produced by the Administration of Sulphur on the Concentration of Certain Sulphur Compounds in the Blood and Urine,* with Lucile Reed, 1927; all published in the *Journal of Biological Chemistry.*

Denis found that the inorganic sulfates of human blood,

as determined by a new method, amounted to 0.5-1.0 milligram per 100 cc. of blood. In nephritis, with nitrogen retention there was also found a retention of inorganic sulfate, as high as 12 to 16 milligrams. When hypertonic solutions of sodium sulfate were injected intravenously into dogs, analysis of the blood, muscles and viscera showed that there was little absorption of the sulfate ion, even though two hours after the injection the sulfate content of the blood was ten times its initial value.

Regarding the distribution of non-protein sulfur in blood, Denis found by determination of the inorganic sulfate, ethereal sulfate and neutral sulfur of the whole blood and plasma in man, dog, beef and goat, that the inorganic sulfates in human blood were contained almost exclusively in the plasma, while with the other three species both inorganic and ethereal sulfates were distributed between corpuscles and plasma. Neutral sulfur in both human and animal blood was present in larger amounts in whole blood, thus indicating its greater concentration in the corpuscles.

That the kidneys exercise a selective action in the excretion of inorganic salts was shown by experiments conducted on dogs and rabbits in which magnesium sulfate, magnesium chloride, sodium sulfate, sodium chloride and sodium phosphate were administered by the intestine and by intravenous injection, subsequent excretion of these salts being followed by means of blood analysis. Particularly noticeable was the selective retention on the part of the kidneys for the sulfate ion, which in one case was found to accumulate in the serum to a value of 3,200 per cent of its initial concentration. Further, a study of the

inorganic constituents of the blood serum in twenty-two cases of nephritis and cardiorenal disease showed a marked increase over normal of the sodium chloride in only four cases, while the inorganic phosphate was increased in ten cases and inorganic sulfates showed increased values in eleven out of seventeen cases. Magnesium and potassium, on the other hand, remained more or less constant, while calcium was found to be decreased in a few cases.

Denis believed that the contrast afforded by the behavior of sodium and chlorine on the one hand, as against phosphate and sulfate on the other, constituted a good demonstration of the specific selective action of the kidney towards the various normal inorganic constituents of serum, somewhat akin to the selective activity known to exist for the various non-protein constituents of blood. "Sodium and chlorine are excreted with great ease even by the damaged kidney; retention of these elements seldom occurs. On the other hand, the sulfate ion is excreted with difficulty, so that in kidney insufficiency the concentration of the sulfate may increase enormously."

When powdered sulfur was added to a standard diet (0.5 gram per kilogram of body weight) Denis found on examination of the urine of animals so fed an average absorption of 10 per cent of the sulfur given. Since sulfur is such an insoluble substance it seemed probable that the observed increases in the total sulfur of the urine were due to oxidation of hydrogen sulfide formed in the intestine by bacterial action on the sulfur. As these increases amounted in some cases to several hundred per cent Denis drew the conclusion that the sulfur normally present in urine probably does not all come from the

intracellular metabolism of protein fragments, but originates in part from oxidation of intestinal hydrogen sulfide.

Finally, mention must be made of the interesting observations of Denis and Borgstrom (1924) on the protein intake of students at Tulane University, as shown by analyses of 24-hour urines covering a period of three years with 233 male students living on their ordinary diet. The average dailv nitrogen excretion of this group was 10.63 grams, which, with an addition of 10 per cent to account for nitrogen lost through the feces, would indicate an average daily consumption of 73.8 grams of protein "much below the average intake recorded for the inhabitants of the United States." Further, it is to be noted that these subjects were at least comfortably supplied with the necessities of life and hence not compelled to restrict their diet in any way. Possibly, as Denis suggested, the high temperature of New Orleans might exert some influence upon the desire for protein food.

During the past few years, another worker at Tulane has been Ralph C. Corley, assistant professor of biochemistry, who has made a special study of *Factors in the Metabolism of Lactose,* investigating particularly the disposal of intravenously administered galactose and the effect of glucose upon it.

At the State College of Kentucky, Kastle and Loevenhart made contributions to physiological chemistry of great importance. Both were men destined to go far in their respective fields, but while at Kentucky they did much to establish the behavior of lipase and especially the reversibility of its action.

Joseph H. Kastle, a graduate of Kentucky State Col-

lege, B.S., 1884, Ph.D., Johns Hopkins University 1888, was professor of chemistry at Kentucky State College from 1888 until 1905, when he became professor of chemistry at the University of Virginia. A vigorous worker, he accomplished much in the study of fermentation, oxidation and reduction in the animal organism, the kinetics of oxidation, oxidases and peroxidases, and auxiliary oxygen carriers.

Arthur S. Loevenhart, a graduate of Kentucky State College in 1898, M.D. Johns Hopkins University 1903, was for some years, 1904-1908, associate and associate professor of physiological chemistry and pharmacology at Johns Hopkins, going in 1908 to the University of Wisconsin as professor of pharmacology and toxicology, where he continued until his death in 1929. While primarily a pharmacologist, Loevenhart did considerable experimental work on catalysis, mechanism of enzyme action and kindred topics.

The first study by Kastle and Loevenhart, *Concerning Lipase, the Fat-splitting Enzyme and the Reversibility of its Action,* published in the *American Chemical Journal* in 1900, was the result of a suggestion by Loevenhart that "possibly lipase might be capable of effecting the synthesis of fats from fatty acid and glycerine as well as their hydrolysis into these products." At that date, there was comparatively little definite knowledge regarding the fat-splitting enzyme and their extensive study brought results of considerable physiological significance, the most important perhaps being that they were able to effect a synthesis of ethyl butyrate from butyric acid

and alcohol, thus proving that the hydrolysis of an ethereal salt by lipase is a reversible reaction.

In studying the hydrolysis of ethereal salts by this enzyme they found that the velocity of the reaction was not proportional to the active mass of the ethereal salt, but was nearly proportional to the concentration of the enzyme. Further, they observed that with ordinary concentrations of the enzyme and the ethereal salt the reaction was incomplete; with very active extracts of lipase, or with very small amounts of ethereal salt, on the other hand, hydrolysis came very close to completion. Finally, as the authors stated "the coefficient of velocity as calculated according to the equation for a reaction of the first order is not constant, but shows a regular falling off as the reaction proceeds."

It is obviously impossible here to give adequate consideration of all the work accomplished by Kastle through his many years of activity in Kentucky and Virginia, but the following studies may be noted as having special biochemical significance: *Phenolphthalein as a Reagent for the Oxidizing Ferments*, with O. M. Shedd, 1901; *On the Nature of Certain of the Oxidizing Ferments*, with A. S. Loevenhart, 1901; *The Inactivity of Lipase Towards the Salts of Certain Acid Ethers Considered in the Light of the Theory of Electrolytic Dissociation*, 1902; *On the Occurrence of Invertase in Plants*, with Mary E. Clark, 1903; *On the Catalytic Decomposition of Hydrogen Peroxide and the Mechanism of Induced Oxidations, Together with a Note of the Nature and Function of Catalase*, with A. S. Loevenhart, two papers 1903-1904; *The Hydrolysis of Ethyl Butyrate by Lipase*, with Marius

E. Johnston and Elias Elvove, 1904; *On the Reduction of Nitrates by Certain Plant Extracts and Metals, and the Accelerating Effect of Certain Substances on the Progress of the Reduction,* with Elias Elvove, 1904; *On the Fate of Potassium Myronate in the Animal Organism and its Hydrolysis by the Ferments of the Liver,* with Eloise C. McCaw, 1904, all published in the *American Chemical Journal.*

In this latter study it was found on examination of a great variety of animals that the liver of all—excepting fish—contains a ferment capable of effecting the hydrolysis of potassium myronate, and that apparently the liver is the only source of animal myrosin. Like other complex carbohydrates and glucosides, potassium myronate is not directly assimilable from the blood but only through the agency of the liver, the process taking place in two stages; the first resulting in the splitting off of glucose, the second in the breaking down of the residue into oil of mustard and potassium acid sulfate.

Kastle and Loevenhart were among the first in this country to study accurately, with the methods then at hand, the oxidation and reduction processes of animal and vegetable organisms. Their work was extensive and followed by results of great physiological value. Among their many conclusions the following may be quoted (1901): "That the oxidation phenomena occurring in the plant, and probably in the animal organism also, can be satisfactorily explained upon the supposition that the readily autoxidizable substances which they contain are oxidized to the peroxide condition by molecular oxygen, and that the peroxides thus formed in turn give up part of

their oxygen to other less oxidizable substances present in the cell. In other words, that the process of rendering oxygen active by the living cell, is probably brought about in essentially the same way that this is accomplished by phosphorus, benzaldehyde and other oxygen carriers, *viz.*, as one phase of autoxidation."

At the school of medicine, University of Louisville, Henry G. Barbour, professor of physiology and pharmacology, aided by several collaborators, has made numerous studies of water exchange in the body, notably changes in the fluid content of blood and plasma, in connection with many physiological and pathological conditions. The following papers may be noted: *The Falling Drop Method for Determining Specific Gravity*, with William F. Hamilton, 1926; *Heat Regulation and Water Exchange*, 1926; *The Effect of Respiratory Gases upon the Density of Blood and Other Fluids*, with William F. Hamilton, 1927; *Retention of Intravenously Injected Fluid in Fasting Anhydremia and Phlorhizin Hydremia*, with Raymond W. Frankmann, 1927.

In order to facilitate the determination of specific gravity where only small quantities of material are available and where the time element is important, Barbour and Hamilton devised a convenient method whereby the result could be obtained with great accuracy, with one drop of body fluid in less than a minute's time. "A 10 cmm. drop of fluid is timed as it falls over a distance of 30 cm. through a mixture of xylene and bromobenzene in a tube of exactly 7.50 mm. bore. Its falling time is compared with that of a 10 cmm. drop of standard $K_2SO_4$ solution of known density. By the use of an alignment chart cor-

recting for room temperature it is possible to calculate the unknown density with an accuracy of $1 \times 10^{-4}$."

Particularly noteworthy are the studies made by Barbour and a number of collaborators on *The Significant Redistribution of Water Between Internal and Surface Tissues and the Blood at the Height of Morphine Withdrawal,* 1929. While with advanced morphine addiction in dogs (daily dose, 30 milligrams per kilogram) there is a tendency towards dehydration of certain internal organs such as the liver, kidney and brain with hydration of the surface tissues (skin, stomach, intestine) and blood, a remarkable redistribution of water was found to occur on sudden withdrawal of the morphine, the effect being most pronounced on the first day, accompanying the characteristic muscle tremors and diarrhea. Internally, there was edema of the brain, muscles, liver and kidney, while the blood, spleen and surface tissues on the other hand showed a considerable loss of water. The heart muscle remained constant, but the pericardial fluid was found to be increased.

Withdrawal of the morphine was also accompanied by a great increase in the environmental water exchange. If the latter subsided early the withdrawal edema likewise ceased, the dehydrated surface tissues and the blood remaining dry. If, however, the high environmental exchange continued, the blood soon tends to show hydration. The results of the many experiments tried plainly indicated that the withdrawal of morphine in addicted animals is accompanied by "muscle tremors, incoördination, decreased blood calcium, edema of the brain, liver and kidneys, anhydremia, diarrhea and disturbed tem-

perature regulation." The degree of brain edema was such as to produce psychic symptoms. Experiments with dogs indicated that the above results were unrelated to nutritional changes; nitrogen absorption, for example, being nearly complete under both addiction and withdrawal of the morphine.

A further study by Barbour, Hunter and Richey may be referred to, *viz., Water Metabolism and Related Changes in Fat-fed and Fat-free-fed Dogs under Morphine Addiction and Acute Withdrawal,* published in the *Journal of Pharmacology and Experimental Therapeutics,* 1929.

At the University of Virginia, Alfred Chanutin (Ph.D. in physiological chemistry, Yale, 1923), associate professor of physiological chemistry, has studied the effect of creatine on growth and its distribution in the tissues, using albino rats as subjects. The average creatine content in normal rats he found to be muscle 0.449 per cent, testes 0.281 per cent, heart 0.174 per cent, brain 0.129 per cent, kidney 0.046 per cent and liver 0.033 per cent. Creatine fed to rats for two months had no effect on the growth curve, and the liver was the only organ that showed a significant increase in creatine on this addition to the diet. When given to man in large doses creatine was completely absorbed from the alimentary tract, without evidence of bacterial decomposition. The creatinine of the urine was increased in amount, the extra creatinine being derived directly from the creatine fed. There was no indirect action on nitrogen metabolism.

In 1924 Glenn E. Cullen became the professor of biochemistry at Vanderbilt University. A graduate of

Michigan University 1912, Ph.D. Columbia University 1917, he was for several years connected with the Rockefeller Institute for Medical Research where he was associated with Donald D. Van Slyke in the study of blood gas and electrolyte equilibrium. Later, 1922-1924, he was associate professor of research medicine at the University of Pennsylvania where he worked on such problems as *The Effect of Ether Anesthesia on the Acid-base Balance of the Blood,* 1922, with several collaborators; *The Initial Acidosis in Anesthesia,* 1923; *The Effect of Insulin Treatment on the Hydrogen-ion Concentration and Alkali Reserve of the Blood in Diabetes,* with Leon Jonas, 1923.

In the department of biochemistry at Vanderbilt University, Cullen's research work has been largely connected with the *Determination of the pH of the Blood,* two papers on this subject appearing in 1927 with Imogene P. Earle. Another worker in biochemistry, in the School of medicine of Vanderbilt University, is J. M. Johlin, who in 1927 while studying *The Effect of Carbon Dioxide Equilibration upon the Surface Tension of Serum,* found that considerable variation in the $CO_2$ tension does not greatly affect the surface tension of the serum.

At the University of Texas, Byron M. Hendrix, professor of biological chemistry in the School of medicine since 1922, has been active in the study of the acid-base balance in the animal body. A graduate of Ohio State University 1909, he had his training in physiological chemistry in the Sheffield Scientific School at Yale, taking the Ph.D. degree in 1915. From 1915 to 1922 he was at the University of Pennsylvania as instructor and then assistant professor of physiological chemistry. Among

Hendrix's later experimental studies the following may be cited: *The Effect of Injections of Sodium Phosphate and Sodium Hippurate upon the Excretion of Acid and Ammonia by the Kidney,* with Jason P. Sanders, 1923; *The Relation of Acidosis and Hyperglucemia to the Excretion of Acids, Bases and Sugar in Uranium Nephritis,* with Meyer Bodansky, 1924; *Alkalosis Produced by Injections of Hydrazine Sulphate in Dogs,* with Ava J. McAmis, 1924; *The Loss of Bases in Diuresis and Its Effects upon the Alkaline Reserve of the Blood,* with Dea B. Calvin, 1925.

The work of Hendrix and his associates showed that injections of disodium phosphate and of sodium hippurate always give rise to an increase of urinary ammonia with evidence that at least a portion of the urinary ammonia is formed in the kidney, in harmony with the view of Benedict and Nash. Further, they found that in uranium nephritis there is some relationship between the degree of acidosis and the glucosuria and hyperglucemia, but the relationship did not appear to be a quantitative one. Again they observed that in the diuresis produced by *sodium chloride, sodium nitrate, sodium sulfate and urea* there is a loss of base through the kidney over and above that lost normally, together with a marked fall in the alkali reserve of the blood. With hydrazine sulfate it was found that injections of this salt in doses of 50 milligrams per kilogram of body weight, using fasting dogs, led within twenty-four hours to the development of alkalosis as shown by increase in pH of the plasma and the $CO_2$-combining power of the whole blood. The alkalosis ap-

peared earlier than the hypoglucemia, but no causal relationship could be demonstrated.

In the University of Missouri physiological chemistry was fathered by Charles W. Greene, a graduate of Stanford University 1892, Ph.D. Johns Hopkins 1898, who as professor of physiology at Missouri since 1900 has conducted many researches of a chemico-physiological character, such as the physiology and chemistry of the Chinook salmon, chemistry of fish ova, and the chemistry of animal tissues in inanition. In 1907 Robert B. Gibson was appointed instructor in physiological chemistry and Louise Stanley instructor in home economics. Both had been trained in physiological chemistry in the Sheffield Scientific School at Yale, Gibson receiving the Ph.D. degree in 1906 and Stanley in 1911. Gibson later became assistant professor of physiological chemistry at the University of Minnesota 1911-1912; professor of physiology in the Philippines 1914-1919; director of the laboratory of clinical chemistry at the University of Iowa since 1919. His experimental work has been varied in character, but with special reference to certain aspects of nutrition and metabolism.

Louise Stanley is one of that rather large group of women trained in the methods and principles of physiological chemistry who have applied their knowledge and experience to the betterment of the economics of home life; especially the application of modern dietary standards to the proper nutrition of the family. After serving as instructor, assistant professor and professor of home economics at the University of Missouri from 1907 to 1923, doing some research work in food chemistry, on

purine enzymes, and with phosphorus compounds, she became in 1923 the chief of the bureau of home economics of the United States Department of Agriculture at Washington.

Albert G. Hogan, a graduate of the University of Missouri in 1907, studied physiological chemistry in the Sheffield Scientific School at Yale, taking the Ph.D. degree in 1914. After a few years as assistant professor of animal nutrition at Kansas College and as professor of biochemistry at the University of Alabama, he became in 1920 the professor of animal nutrition at the University of Missouri. His experimental work has been directed especially to growth and reproduction as affected by nutrition, his studies with several collaborators *Concerning the Adequacy of Synthetic Diets for the Growth of the Chick,* 1925, and *Acceleration of Growth by Dietary Modifications,* 1928, being especially noteworthy.

Turning to the University of Iowa, the influence of Elbert W. Rockwood, professor of chemistry and toxicology at that university since 1888, has been a potent factor in the gradual development of physiological chemistry there. A graduate of Amherst College in 1884, Rockwood soon manifested an interest in the applications of chemistry to physiology which led to successive periods of study at the universities of Göttingen, Strassburg and Leipzig during 1889 to 1894. Later, he spent some time in the Sheffield laboratory of physiological chemistry at Yale, taking the Ph.D. degree there in 1904. Many researches have come from his laboratory dealing with such subjects as uric acid, enzyme action, mechanism of the action of amino-acid promoters upon enzymes, etc. As

noted previously, Victor C. Myers was for a time head of the department of biochemistry at Iowa 1924-1927, while Robert B. Gibson has been active there in the field of clinical chemistry since 1919. In 1928 Henry A. Mattill became the professor of biochemistry.

Mattill, a graduate of Western Reserve, 1906, Ph.D. University of Illinois, 1910, served for a time as assistant professor and finally as professor of physiological chemistry at the University of Utah, 1910-1915; assistant professor of nutrition at the University of California, 1915-1919; professor of biochemistry at the University of Rochester from 1919 until his appointment at Iowa. He has been particularly interested in the study of diet and reproduction, especially with reference to the influence of certain vitamines, as *The Utilization of Carbohydrate by Rats Deprived of Vitamine B,* 1923, and *Vitamine E and Reproduction on Synthetic and Milk Diets,* with M. M. Clayton, 1926. In the latter study, observations on 150 animals fed various synthetic rations indicated that vitamine E is a necessary dietary constituent for the establishment of fertility in male rats. With female animals it was found that on similar rations free from vitamine E there was exhibited the same type of sterility as is encountered in animals fed on high fat, milk rations.

Still another worker in physiological chemistry at the University of Iowa is Amy L. Daniels, research professor of nutrition, child welfare research station. She and the position she occupies afford another illustration of the growth in this country of the movement for the improvement of the health of the community through application of the most recent findings in the study of nutrition. A

graduate of Columbia University, 1906, a student of physiological chemistry at the Sheffield Scientific School, taking the Ph.D degree in 1912, she served for a time as assistant professor of home economics at the University of Missouri, later as associate professor and professor of nutrition at the University of Wisconsin 1914-1918, after which she went to the University of Iowa.

Among her various scientific studies the following may be cited: *The Nutritive Value of the Soy Bean*, with Nell B. Nichols, 1917; *Influence of High Temperature and Dilute Alkalies on the Antineuritic Properties of Foods*, with Nellie I. McClurg, 1918; *The Effect of Heat Treatment of Milk Feedings on the Mineral Metabolism of Infants*, with Genevieve Stearns, 1924; all published in the *Journal of Biological Chemistry.*

The yellow soy bean, plainly one of the most valuable of the leguminous seeds, was shown not only to contain a high percentage of physiologically good protein, with fat and carbohydrate sufficient to furnish a considerable amount of energy, but there was also present a fat-soluble food accessory and water-soluble growth determinant. To render the bean a more nearly complete food, however, there is need for the addition of sodium chloride and calcium compounds.

By feeding experiments with cooked foods, with and without the addition of sodium bicarbonate, and studying the curves of growth, it was found that neither high temperatures nor dilute alkalies at boiling temperature were detrimental to the antineuritic vitamine in foods. In studying the effects of heat treatment of milk it was observed that when the milk mixtures were quickly boiled

the infants in some cases gained over the results obtained when milk mixtures pasteurized by the "hold" method were fed.

At the medical school of Northwestern University, John H. Long, professor of chemistry there from 1881 until his death in 1918, was active in physiological chemistry, both in teaching and in research. A graduate of the University of Kansas, 1877, he had the advantage of extended study in Germany, at Breslau, Würzburg and Tübingen, taking the degree of Sc.D. at the latter university in 1879. He served as chemist on the State Board of Health of Illinois for many years, doing much work on problems connected with the purification of water supplies. He was a member of the Referee Board of consulting scientific experts appointed by President Roosevelt to aid the United States Department of Agriculture, and in his laboratory at Chicago were conducted many of the experimental studies bearing on the physiological action of various food preservatives carried on by the board. He combined rare good judgment with the exact knowledge of the trained scientific mind and hence he was especially useful in the solution of many problems connected with the public health. Of his more strictly scientific work, his studies of the optical rotation of organic substances, the action of digestive enzymes, and the character of the salts of casein are most noteworthy from a chemico-physiological standpoint.

At present, George L. Foster occupies the position of assistant professor of biochemistry at Northwestern University Medical School. A graduate of Dartmouth College, 1913, Ph.D. of Harvard University, 1921, he was

for several years instructor and then assistant professor of biological chemistry at the University of California.

At the University of Nebraska College of Medicine, Sergius Morgulis has been the professor of biochemistry since 1922. After his early training in Russia he came under the influence of Folin and Benedict, taking the Ph.D. degree at Harvard in 1910 and during the years 1913-1916 he was instructor and associate in biochemistry at Columbia University. From 1916 to 1922 he was professor of biochemistry and physiology at Creighton University, Omaha. A very active worker, Morgulis has made many contributions to knowledge, especially his *Studies on the Effect of Temperature on the Catalase Reaction,* with M. Beber and I. Rabkin, 1926-1928, in which such problems as *Heat Inactivation of Catalase at Different Hydrogen-ion Concentrations; Effect of Different Hydrogen Peroxide Concentrations;* and *Loss of Catalase Activity* were carefully studied.

Among other lines of work his experimental study of the *Blood Changes During Digestion, with Special Reference to Urea Formation,* with several collaborators, 1925, may be referred to; one conclusion reached being that what is ordinarily designated as "urea formation" may not represent a single process, but that the deamination of the amino-acids and the synthesis of urea may be independent processes. Again, his studies on fasting animals have yielded results of considerable physiological importance, while his book on *Fasting and Undernutrition,* 1923, presents a good résumé of his own views and those of other workers in the same field.

At Ohio State University, John F. Lyman in 1909 be-

came associate professor and in 1915 professor of agricultural chemistry, where he has been occupied with experimental work in both agricultural chemistry and biochemistry. Trained in physiological chemistry at the Sheffield Scientific School, Yale, where he took the degree of Ph.D. in 1909, he has worked especially on the *Metabolism of Fats*, 1917, and on the *Effect of High Protein Acid-forming Diets on the Excretion of Ammonia by Rabbits*, 1919.

In studies on the utilization of palmitic acid, glyceryl palmitate and ethyl palmitate by the dog he found the following utilization values: lard 96.6 per cent, ethyl palmitate 58 per cent, glyceryl palmitate 95 per cent, palmitic acid 82 per cent. Emulsified esters of the fatty acids he found were not absorbed as such, but absorption was limited by the rate of hydrolysis. Apparently, the melting point of the ester is not the only factor, probably not the chief factor, in determining the rate of hydrolysis and absorption. Neither free palmitic acid nor ethyl palmitate were stored in appreciable amounts in the fat depots when fed to white rats, the main deposit being tripalmitin. When a fat-poor diet was fed, the fat deposited was found to be markedly different from that laid down when the diet contains palmitic acid or its esters.

Working with Bernard Raymond, Lyman found that rabbits fed high protein diets in which the acid-forming elements predominate excrete a distinctly acid urine containing appreciable quantities of ammonia. Ammonia, however, as a factor in the acid-base equilibrium of the rabbit, in contrast to some other herbivora, is relatively unimportant even under conditions of sup-

posedly severe acid intoxication. Addition of sodium citrate to a high protein, acid-producing diet caused in the rabbit a marked fall in urinary acidity and when administered in sufficient quantity led to the disappearance of urinary ammonia. Noteworthy is the fact that when ammonium lactate in small quantity is added to a milk diet there is a fall rather than a rise in ammonia excretion in the rabbit, indicating that the failure of this animal to utilize ammonia to a greater extent for neutralization purposes is due not so much to a deficiency of ammonia in the organism as to other causes.

At the Merrill-Palmer school of Detroit, Icie G. Macy, in charge of nutrition research, also associate in nutrition at the children's hospital of Michigan, has done much work of a biochemical character in the field of nutrition. A graduate of Chicago University, B.S., 1916, she studied physiological chemistry at the Sheffield Scientific School at Yale, taking the Ph.D. degree in 1920, assuming the position at Detroit in 1923. Among her various studies attention may be called to the work on human milk, especially the vitamine content, which was published in 1927 in the *Journal of Biological Chemistry;* a series of papers with several collaborators, notably Julia Outhouse, Alice Graham and M. Louise Long.

Employing rats as experimental animals, with a standardized technique for the quantitative estimation of the vitamine content of human milk and a purified experimental ration adequate for the maintenance of rats, it was found that milk from women living on the average American dietary is a relatively rich source of vitamine A, 2.5-3.0 cc. of mixed milk from a group of wet nurses

in various stages of lactation being sufficient to satisfy the nutritive demands for this factor during growth and reproduction of the rat. The experimental data obtained made it quite clear that the vitamine A content of breast milk is a most important factor in influencing the economy of nutrition during the periods of most rapid growth, *i.e.*, fetal, infancy and childhood. Lactation requirements for vitamine B appeared to be 3 to 5 times greater than for growth, while the demand for vitamine E was not as clearly defined. With cow's milk, while 3 cc. of fresh raw milk fed daily appeared to contain sufficient vitamine A to produce satisfactory growth in rats, this amount was not always sufficient to protect against secondary pathological conditions.

As is well understood, diets low in vitamines A and B are closely associated with a child's susceptibility to infections, as cases in children's clinics frequently indicate and since the vitamine concentration of milk is dependent upon the vitamines ingested in the food of the mother—the maternal organism being unable to synthesize these factors—it becomes imperative that they be supplied in adequate quantity. The results of the experiments reported by Miss Macy and her co-workers suggest that many mothers do not furnish enough of vitamine B to meet the needs of their babies. "At the best, the average healthy mother is producing a milk that is exceedingly low in vitamine B, and for the economy of the mother's nutrition and to safeguard her offspring food materials rich in important food components should form a prominent part in the diet of pregnant and lactating women."

In 1903 John J. R. Macleod became the professor of

physiology at Western Reserve University, where he served for fifteen years, going then to the University of Toronto and later to the University of Aberdeen, where he had graduated in 1898. Broadly trained in physiology he was well versed in the principles and methods of physiological chemistry and had served as lecturer on biochemistry in London, 1902.

His first chemical studies in this country had to do with carbamates, two papers: *The Quantitative Estimation of Carbamates;* and *Contributions to Our Knowledge of the Chemistry of Carbamates,* appearing from his laboratory in 1905 in collaboration with Howard D. Haskins, who later, in 1915, became the professor of biochemistry at the University of Oregon. This work was followed by a long series of studies extending through many years, under the general title of *Studies in Experimental Glycosuria;* all published in the *American Journal of Physiology.*

The earlier papers by Macleod were more largely physiological than chemical, the first in 1907 having the title *On the Existence of Afferent and Efferent Nerve Fibers Controlling the Amount of Sugar in the Blood;* while the second paper of the series, appearing in 1908, was entitled *Some Experiments Bearing on the Nature of the Glycogenolytic Fibers in the Great Splanchnic Nerve.* The fourth paper, 1909, dealt with *The Cause of the Hyperglycaemia Produced by Asphyxia,* in which he showed that the increased amount of reducing substance in the blood in asphyxia and curare poisoning comes from the glycogen of the liver, removal of the liver from the circulation being followed by hypoglycemia both in nor-

mal animals and in animals asphyxiated or injected with curare. He found further that the asphyxial blood acts directly on the hepatic cells and not through the intermediation of the central nervous system, for the usual hyperglycemia followed asphyxiation after all the hepatic nerves were cut. Finally, the conclusion was reached that the stimulation of hepatic glycogenolysis which accompanies asphyxia in the intact animals is due to the excess of carbon dioxide in the blood and not to the deficiency of oxygen.

Other studies in this same series that may be noted are *The Distribution of Glycogenolytic Ferment in the Animal Body, Especially of the Dog,* with R. G. Pearce, 1910; *The Distribution of Glycogen over the Liver under Various Conditions, Post Mortem Glycogenolysis,* with R. G. Pearce, 1911; *The Relationship of the Adrenal Glands to Sugar Production by the Liver,* with R. G. Pearce, 1912; *Blood Glycolysis; Its Extent and Significance in Carbohydrate Metabolism, The Supposed Existence of "Sucre Virtuel" in Freshly Drawn Blood,* 1913. This last paper was published in the *Journal of Biological Chemistry.*

Among the many conclusions drawn from the experimental data collected the following may be cited. Comparison of the glycogenolytic activity of extracts (made by use of the Buchner press) of blood-free organs and tissues and of blood serum, showed that by far the largest amount of glycogenase is present in the pancreas, the serum and liver coming next, while the kidneys, intestines and muscles always contain less of the endoenzyme than the blood serum. Extracts of the blood-free liver of the

dog, pig, rabbit and lamb compared with the blood serum revealed that the largest amounts of glycogenase are contained in the tissues of the dog and pig, while the tissues of the two herbivorous animals contain far less of the enzyme. Further, the results collectively indicated quite clearly that the glycogenolytic power of the blood serum is markedly greater than that of the blood-free liver.

In the experiments with the adrenal glands, the amount of reducing substance in the blood removed from the vena cava opposite the liver was compared under such conditions as would demonstrate the relationship of these glands to the process of hepatic glycogenolysis. It was found that after excision of the left adrenal gland, stimulation of the great splanchnic nerve on the same side did not lead to hyperglycogenolysis, such as usually follows the stimulation of this nerve. Further, it was found that stimulation of the hepatic plexus caused marked hyperglycemia under normal conditions, but if the adrenal glands were removed then hyperglycemia failed to result on stimulation of the plexus.

If adrenaline was injected into the portal vein, after excision of the hepatic plexus, hyperglycemia was found *to occur, thus indicating that adrenaline has the power to* excite the local glycogenolytic mechanism. Finally, as stated by Macleod "only when the adrenal glands are intact is it possible, by stimulation of the nerves supplying the liver, to excite hyperglycogenolysis. Some influence exercised by the adrenal glands is evidently essential for the functional integrity of the nerves which control the process of glycogenolysis."

In his study of blood glycolysis Macleod found that this

function is associated solely with the corpuscles, the serum lacking such power. Frequent washing of the corpuscles with isotonic saline caused the glycolytic power to disappear. Addition of dextrose to blood did not materially increase the extent of the glycolysis occurring in a given time. In other words, the absolute amount of dextrose destroyed *in vitro* when the blood is incubated for a given period is quite independent of the original concentration of the sugar.

Macleod drew the general conclusion from his experiments that "the glycolysis which we study in blood *in vitro* does not bear any important relationship to the glycolysis which occurs in the intact animal. The amount of sugar which disappears from blood *in vitro* is by far too small to account for more than a minute fraction of that which disappears in the body." Finally, "the glycolysis which occurs in blood is most probably of no importance in carbohydrate metabolism." It does not require much imagination to see in these and other related studies which came from Macleod's laboratory a suggestive prelude to the later work on insulin, for which in 1924 he received with Frederick G. Banting the Nobel prize.

At the University of California, T. Brailsford Robertson was for many years an active worker in the fields of physiology and biochemistry. A graduate of the University of Adelaide, South Australia, B.S., 1905, Ph.D., University of California, 1907, he became eventually professor of biochemistry and pharmacology at the latter university, holding that position until he was appointed professor of physiology and biochemistry at Adelaide. Interested in physical chemistry, especially in its applica

tions to physiology, he devoted much time to experimental study of the physical chemistry of the proteins, to experimental studies on growth, and to many allied topics of physiological importance. His contributions from the Rudolph Spreckels Physiological Laboratory of the University of California were both numerous and varied in character, published largely in the *Journal of Biological Chemistry* and in the *Journal of Physical Chemistry.*

Among his many studies the following may be cited: *On the Synthesis of Paranuclein Through the Agency of Pepsin and the Chemical Mechanics of the Hydrolysis and Synthesis of Proteins Through the Agency of Enzymes,* 1908, which led to his hypothesis of "reciprocal catalysis"; *On the Nature of the Chemical Mechanism which Maintains the Neutrality of the Tissues and the Tissue-Fluids,* 1909; *Concerning the Relative Magnitude of the Parts Played by the Proteins and by the Bicarbonates in the Maintenance of the Neutrality of the Blood,* 1910; *Note on the Applicability of the Law of Amphoteric Electrolytes to Serum Globulin,* 1908; *On the Composition of Certain Substances Produced by the Action of Pepsin upon the Products of the Complete Hydrolysis of Casein,* 1911; *The Preparation and Properties of a Compound Protein, Globin Caseinate,* 1912; *Studies in the Blood Relationship of Animals as Displayed in the Composition of the Serum Proteins,* 1912; *On the Refractive Indices of Solutions of Certain Proteins,* some eight individual studies, 1908-1912; *The Influence of Alkali and Alkali Earth Salts upon the Rate of Solution of Casein by Sodium Hydroxide,* with K. Miyake, 1916. In 1918, appeared the book *The Physical Chemistry of*

*the Proteins,* in which Robertson presented a résumé of existing knowledge on the subject with expression of his own views.

Robertson's *Experimental Studies on Growth* have covered a wide range of topics, such as the *Chemical Mechanics of Cell-division; The Isolation and Properties of Tethelin, the Growth-controlling Principle of the Anterior Lobe of the Pituitary Body; The Influence of Lecithin upon the Growth of the White Mouse; The Influence of Lecithin and Cholesterin upon the Growth of Tumors; The Influence of Brain Tissue, Freed from Cholesterol, upon the Growth of the White Mouse.* In 1923, appeared his book, *The Chemical Basis of Growth and Senescence,* one of the monographs on experimental biology, edited by Jacques Loeb, T. H. Morgan and W. J. V. Osterhout.

Carl L. A. Schmidt has been professor of biochemistry and pharmacology at the University of California since 1924. A graduate of that university, Ph.D., 1916, he has been associated with the instruction there since 1915, his research work being largely in connection with proteins and amino-acids. Edward S. Sundstroem, assistant professor of biochemistry at California since 1922, M.D., Helsingfors 1910, has been especially active in the study of the *Physiological Effects of Tropical Climate,* his reports in the *University of California Publications in Physiology,* 1926, being particularly noteworthy.

Another worker at the University of California whose accomplishments in the field of biochemistry merit special attention is Herbert M. Evans, professor of anatomy since 1915. A Graduate of California, 1904, M.D., Johns Hop-

kins 1908, instructor and ultimately associate professor of anatomy in the latter university 1908-1915, he has done much in the study of endocrinology, his discovery (1922) of a fertility vitamine in various foods such as meat, lettuce, oats, alfalfa and others being especially important. Certain aspects of his work will be referred to in later chapters.

Martha R. Jones, Ph.D. in physiological chemistry, Sheffield Scientific School at Yale, 1920, occupied for several years the position of research associate in pediatrics at the medical school of California University, and Helen B. Thompson, Ph.D. in physiological chemistry, Sheffield Scientific School, 1917, is professor of home economics at the Southern branch of the University of California, both active in the study of problems connected with nutrition. Ruth E. Okey, Ph.D., University of Illinois, 1918, has been assistant and associate professor of household science at the University of California since 1919, where she has applied her knowledge of the physiology of nutrition to the needs of the family and at the same time carried on research work on blood and certain aspects of metabolism.

At Stanford University Robert E. Swain has been the professor of chemistry since 1912. A graduate of Stanford, 1899, Ph.D. in physiological chemistry at the Sheffield Scientific School 1904, a student at Heidelberg and Strassburg, he has been active in the study of various problems of chemico-physiological importance, notably the formation of kynurenic acid in the animal body, and the physiological action of thallium salts. James M. Luck is the assistant professor of biochemistry.

As a center for chemico-physiological research, Stanford enjoys the advantage of the presence of the *Food Research Institute* established in 1921 with Carl L. Alsberg and Alonzo E. Taylor as directors, both eminent in the field of biochemistry.

In the school of medicine of the University of Colorado, Robert C. Lewis, Ph.D., Sheffield Scientific School at Yale, 1912, after some years of service as biochemist of the United States Public Health Service, became the professor of biochemistry where he has been active in the study of blood chemistry and some phases of metabolism.

From the physiological chemistry laboratory of the University of Indiana appeared in 1922 an interesting study entitled *The Tryptophane Content of Some Proteins*, by Clarence E. May and Embree R. Rose, published in the *Journal of Biological Chemistry.* Clarence E. May, a graduate of the University of Indiana, 1904, Ph.D., Columbia University 1908, has been assistant professor and later professor of chemistry at Indiana since 1908.

Turning again to the University of Illinois mention must be made of two workers in physiological chemistry, both of whom bear the title professor of animal nutrition, *viz.*, Harry S. Grindley and Harold H. Mitchell. Each has accomplished much experimental work, especially in connection with the nutrition of farm animals. Grindley, a graduate of Illinois 1888, Sc.D., Harvard 1894, became assistant professor at Illinois in 1894 and professor in 1907. Mitchell, likewise a graduate of Illinois 1909, Ph.D., 1915, has ever since been associated with the

work of the experiment station at that university in the field of animal husbandry.

Among Mitchell's many investigations of more general biochemical interest reference may be made to the following: *Feeding Experiments on the Substitution of Protein by Definite Mixtures of Isolated Amino-acids*, 1916; *The Influence of Protein Feeding on the Concentration of Amino-acids and Their Nitrogenous Metabolites in the Tissues*, 1918; *The Relation Between the Endogenous Catabolism and the Non-protein Constituents of the Tissues*, with W. B. Nevins and F. E. Kendall, 1922; *A Method of Determining the Biological Value of Protein*, 1923; *The Biological Value of Proteins at Different Levels of Intake*, 1923; *The Supplementary Relations Among Proteins*, 1923; *The Biological Value for Maintenance and Growth of the Proteins of Whole Wheat, Eggs and Pork*, with G. G. Carman, 1924; *The Effect of Muscular Work upon the Endogenous Catabolism of the Tissues*, with J. H. Kruger, 1927; all published in the *Journal of Biological Chemistry*. Mitchell's recent book, 1929, *The Biochemistry of the Amino-acids*, written in conjunction with T. S. Hamilton, associate in animal nutrition at the College of Agriculture, University of Illinois, one of the American Chemical Society monographs, gives an illuminating presentation of present-day knowledge of the subject.

Grindley has been much occupied with studies of the physiological action of food preservatives, notably the "investigation of the influence of saltpeter on the nutrition and health of man with reference to its occurrence in cured meats" conducted in the laboratory of physio-

logical chemistry, department of animal husbandry, of the University of Illinois, by Harry S. Grindley, Ward J. MacNeal, Harold H. Mitchell and numerous other collaborators with the coöperation of an Advisory Board composed of Theobald Smith of the Rockefeller Institute for Medical Research, Albert P. Mathews of the University of Çincinnati, David L. Edsall of the Harvard University Medical School, Harry S. Grindley and Russell H. Chittenden, the elaborate and detailed report of the work being published in five volumes, 1918-1929.

# CHAPTER X

Physiological chemistry at Yale—Physiological chemistry as a biological science—Relation to medicine—Research work in physiological chemistry—Early studies by Chittenden and collaborators—Experimental work of Lafayette B. Mendel, Frank P. Underhill, Arthur H. Smith, George R. Cowgill, Hubert B. Vickery.

The laboratory of physiological chemistry at Yale; which as stated in an earlier chapter had its beginning in the Sheffield Scientific School in 1874, has during its fifty-five years of existence shown steady growth as a center both for instruction and research. From 1874 until 1903 the responsibility for the laboratory and its activities rested wholly with the writer, aided by numerous instructors and assistants who naturally changed from year to year as they passed on to positions of greater importance.

In 1897, as previously noted, Lafayette B. Mendel was made assistant professor and in 1903 professor of physiological chemistry in the Sheffield Scientific School, from which date he shared to the fullest extent in the control of this department of study. In 1922, on the retirement of Chittenden from active service, Mendel became the head of the department and solely responsible for the conduct of its affairs. Long before this date, however, due to the conditions which prevailed—the duties as director of the Sheffield Scientific School consuming a large proportion of Chittenden's time—Mendel had in

reality carried the major part of the burden of instruction and guidance in research, and to him must be ascribed a full measure of credit for such standing as the laboratory has attained during the later years.

Another force in the Sheffield laboratory for a long period was Frank P. Underhill, instructor 1903-1907 and assistant professor of physiological chemistry 1907-1918. A graduate of the Sheffield Scientific School, 1900, Ph.D., 1903, Underhill through his ability as a teacher and his activities in research did much to uphold the scientific standing of the laboratory, many important contributions to knowledge coming from his own efforts and from the efforts of others working under his guidance. In 1912 he was appointed professor of pathological chemistry in the Yale Medical School, retaining, however, his position in the Sheffield Scientific School where the larger proportion of his work was carried on until the college year 1918-1919. On the reorganization of the University in 1921, Underhill became the professor of pharmacology and toxicology in the Yale Medical School.

It may not be amiss here, as a matter of some historical interest, to recall the fact that physiological chemistry at Yale up to the reorganization of the University in 1921 had been entirely under the jurisdiction of the school of science. The opinion prevailed that physiological chemistry should be considered as a biological science in the broadest sense of the term, as much so as zoölogy, comparative anatomy or physiology, ready however to give its aid in any direction that might be called for but not to be linked indissolubly with the science or art of medicine.

There was a time in the earlier history of medicine when the devotees of chemistry, zoölogy and botany were largely medical men, for they, almost alone at that period, were able to recognize the scientific importance of these subjects and their possible applications. As these sciences grew however, it became clearly manifest that too close union with medicine would only serve to retard their development, that a progressive science if it is to have opportunity for expansion and freedom for full usefulness must occupy an independent position unhampered by specific requirements or restrictions.

Though the student and practitioner of medicine today must of necessity be fairly well trained in chemistry, indeed must depend upon chemistry for much knowledge of which he stands in need, yet no one in this generation would think of advocating the establishment of chairs of chemistry in a medical school. Such a procedure would truly be archaic. Wherein is physiological chemistry in any different position? It is true that physiological chemistry, or biochemistry if that term is preferred, is giving first aid in the solving of many problems of great physiological and pathological importance, but so too are chemistry and physics. It may be said that many problems of a chemical nature present themselves which only the well-trained medical man can appreciate the bearings of, but if such problems are to be solved accurately the one undertaking the investigation must be equally well trained in chemistry, otherwise failure is bound to result. It is certainly as practical for the well-trained physiological chemist to cope with such problems, through study of the special medical aspects of the case, as for the medical man

to acquire the necessary knowledge of and experience in the use of the chemical procedures called for.

The main point however is that physiological chemistry should be recognized and treated as a pure science unhampered in its growth by any form of application. A science, whether it be biological or physical, to undergo a well-rounded development should have perfect freedom to progress and expand in any and all directions without regard to possible applications. Applications will come fast enough as the science advances, but just so soon as a science feels the pressure of an influence tending to limit its activities to any given channel then there is danger of a one-sided development with a restraining effect upon the growth of the science as a whole. This is a danger, as the writer sees it, which threatens the broad development of physiological chemistry in this country.

The Sheffield Laboratory of Physiological Chemistry had always had a justifiable feeling of pride that its establishment had come about through the development of a broad course of instruction in biology and had cherished the hope that as the years of its growth increased it would continue to stand as a representative of an idea or principle biologically sound. There was encouragement also in the fact that at the two great educational centers of England, *viz.*, the universities of Oxford and Cambridge, the development of physiological chemistry had taken place not as a recognized part of the medical curriculum but as an independent part or unit of the university. And certainly at the University of Cambridge especially, under the inspiring leadership of Sir Frederick Hopkins, there can be no criticism of the great importance of the work ac-

complished there; work which has not only contributed results of broad physiological value, but has rendered distinct aid to medicine.

That the medical schools of America have contributed largely to the development of the science in this country the record presented in the preceding pages fully shows, but this fact does not in my judgment weaken the view I am disposed to hold. There is a large and growing demand for well-trained men in physiological chemistry; men grounded in the principles of physics, chemistry, especially organic chemistry, physiology and general biology. They should come up through the graduate school of the university where they breathe an atmosphere of pure science, and where all the sciences are on the same level, and not through a medical school where of necessity the atmosphere and the surroundings are properly such as pertain to a school especially charged with the training of candidates for the practice of medicine.

Physiological chemistry as one of the biological sciences on which medicine rests belongs not primarily in the medical schools but with the general courses of the university, where it will be free to expand and develop along the broadest possible lines. That the writer is not alone in this opinion is indicated by the following quotation from the president [1] of Stanford University:

"The time is about ripe for a readjustment of the relationship of medical instruction to the fundamental sciences on which it rests. Not many years ago physics and chemistry were included as part of the medical curriculum. At the present time anatomy including histology,

[1] Ray Lyman Wilbur, M.D., *J. Am. Med. Assoc.*, 88: March 5 (1927).

neurology and embryology, physiology, physiological chemistry and bacteriology are included in some degree in all medical schools. In fact, there has been a steady insistence on the part of the medical profession that these basic sciences belong in the medical curriculum and nowhere else. This attitude has been a definite obstacle to the advance of physiology and anatomy in America. . . . These basic sciences need to be set free from the limited claims of the medical curriculum. The men in them need to go into their respective fields in the broadest possible way, using all related information in the hope that advances may be made which can later be brought into medicine. America has been distinctively defective in its research work in the fundamental sciences. The limitations of the various departments by the medical school has played its part in this unfortunate condition."

An analogous position presents itself in connection with the engineering schools of the country, especially electrical and mechanical engineering. Physics is the fundamental science upon which these two engineering professions rest and without the applications of physics the progress in engineering so conspicuous in our day would have been impossible, yet would any right-minded person consider it in any sense wise to place the physics departments of our universities and scientific schools under the jurisdiction of the engineering schools? Would not such a procedure be distinctly detrimental to the growth and development of physics?

That the medical schools of the country have great need of the teachings of physiological chemistry no one will deny, least of all the writer, but to place it in the

medical schools on the same plane as pharmacology, pathology, pediatrics and internal medicine, gives it the stamp of a specialized medical science, sure in the long run to interfere with its standing and development as a basic biological science.

What the medical schools should do to meet their special needs, as the writer sees it, is to develop a department of *pathological chemistry,* especially adapted to the requirements in clinical medicine, pediatrics and public health, with special reference to the technique of clinical chemistry, the methods to be followed in a study of the diseases of metabolism and in the study of foods and nutrition as applied to public health, without attempting to assume the responsibility for the development of a science which is broad enough and large enough to stand alone.

In view of the opinions given it was a source of regret to many when in 1923 the Sheffield Laboratory of Physiological Chemistry at Yale, after forty-nine years of fruitful development, was transferred to the new Sterling Hall of Medicine. To be sure, increased laboratory facilities and improved conditions generally offer some compensation, but whether these will in the long run equal the many advantages which pertain to a recognized and established position as an independent unit in a group of biological sciences, all free from any hampering restrictions, only time can determine. As stated in another connection, Mendel in 1921 was made Sterling Professor of Physiological Chemistry in Yale University.

In 1929 the staff of the department consisted, in addition to Mendel, of Arthur H. Smith and George R. Cow-

gill, assistant professors of physiological chemistry, Hubert B. Vickery, lecturer on the chemistry of proteins, William E. Anderson, research assistant, Harry M. Vars, instructor, and five assistants. All this stands for development in physiological chemistry and as such properly belongs in the record.

The research work carried on in the laboratory has covered a wide range, necessarily so owing to the large numbers who have had their training at New Haven. The total number of titles covering studies from the laboratory during the years 1880-1927 is five hundred and forty, the earlier articles having been published in the *American Chemical Journal*, the *Transactions of the Connecticut Academy, The Journal of Experimental Medicine,* and the *Journal of Physiology.* After the establishment of the *American Journal of Physiology* and the *Journal of Biological Chemistry* the work from the laboratory appeared largely in these two publications.

As stated in Chapter II, a volume of *Studies in Physiological Chemistry* was issued in 1885, composed of reprints of the papers published during the years 1884-1885. Three such volumes appeared up to 1890 edited by Chittenden; from 1901 to 1907 two additional volumes appeared edited by Chittenden and Mendel, and from 1907 to 1918 five volumes edited by Chittenden, Mendel and Underhill. Covering the work of 1919-1921 one volume was issued edited by Chittenden, Mendel, Smith and Cowgill, all these volumes bearing the imprint *The Laboratory of Physiological Chemistry, Sheffield Scientific School, Yale University.* The next two volumes, for the years 1922-1924 and 1925-1927, were edited by Men-

del, Smith and Cowgill, and bear the imprint *The Laboratory of Physiological Chemistry, Sterling Hall of Medicine, Yale University*. To give any adequate description of the contents of these volumes is obviously quite impossible here, but a few titles with the names of the authors may have some significance.

Of studies by Chittenden and various collaborators during the earlier years the following may be cited: *On the Detection and Determination of Arsenic in Organic Matter*, with Henry H. Donaldson, 1880; *On Arsenical Bismuth-subnitrate*, with Samuel W. Lambert, 1882; *On the Alkalinity and Diastatic Action of Human Saliva*, with John S. Ely, 1883; *The Post-mortem Formation of Sugar in the Liver in the Presence of Peptones*, with Alexander Lambert, 1885; *The Diastatic Action of Saliva as Modified by Various Conditions, Studied Quantitatively*, with Herbert E. Smith, 1885; *Influence of Potassium and Ammonium Bromides on Metabolism*, with William L. Culbert, 1885; *Some Experiments on the Physiological Action of Uranium Salts*, with Alexander Lambert, 1888; *Some Experiments on the Influence of Arsenic and Antimony on Glycogenic Function and Fatty Degeneration of the Liver*, with Joseph A. Blake, 1888; *The Relative Absorption of Nickel and Cobalt*, with Charles Norris, 1888; *The Relative Formation of Proteoses and Peptones in Gastric Digestion*, with John A. Hartwell, 1891; *The Influence of Alcohol on Proteid Metabolism*, 1891; *A Study of the Proteids of the Corn or Maize Kernel*, with Thomas B. Osborne, 1891; *On the Proteolytic Action of Bromelin, the Ferment of Pineapple Juice*, with T. Stuart Hart and Theodore C. Janeway, 1894; *A Study of the*

*Primary Cleavage Products Resulting from the Action of Superheated Water on Coagulated Egg-albumin*, with Frank S. Meara, 1894; *On the Proteolysis of Crystallized Globulin*, with Lafayette B. Mendel, 1894; *Variations in the Amylolytic Power and Chemical Composition of Human Mixed Saliva*, with Alfred N. Richards, 1898; *A Chemico-physiological Study of Certain Derivatives of the Proteids*, with Lafayette B. Mendel and Yandell Henderson, 1899; *The Production in Dogs of a Pathological Condition Which Closely Resembles Human Pellagra*, with Frank P. Underhill, 1917.

In attempting a brief outline of the experimental work carried on by Mendel it is necessary to stress the fact that the large number of advanced students in the laboratory called for many different lines of research in order to meet individual needs. Hence, many studies not directly connected with Mendel's chief activities were pursued with the collaboration of students who had reached the research stage.

Such studies may be indicated by the following list: *The Excretion of Kynurenic Acid*, with Edward C. Schneider, 1901; *Observations on a Case of Cyclic Albuminuria*, with Donald R. Hooker, 1901; *Some Decomposition Products of the Crystallized Vegetable Proteid Edestin*, with Phoebus A. Levene, 1901; *Experimental Studies on the Physiology of the Molluscs*, four papers, with Harold C. Bradley and H. Gideon Wells, 1905-1906; *On Absorption from the Peritoneal Cavity*, with H. Gideon Wells, 1907; *The Physiological Utilization of Some Complex Carbohydrates*, with Mary D. Swartz, 1910; *The Metabolism of Some Pyrimidine De-*

*rivatives,* with Victor C. Myers, 1910; *Studies in Nutrition, Utilization of Different Proteins,* six papers, with Morris S. Fine, 1911-1912; *The Question of Fat Absorption from the Mammalian Stomach,* with Emil J. Baumann, 1915; *The Rôle of the Digestive Glands in the Excretion of Endogenous Uric Acid,* with Raymond L. Stehle, 1915; *Comparative Studies of the Physiological Value and Toxicity of Cotton Seed and Some of its Products,* with Icie G. Macy, 1920; *Experiments on the Metabolism of Thymine,* with Harry J. Deuel, Jr., 1923; *Protein Fevers,* with Florence B. Seibert, 1923.

Mendel's main experimental work, however, is to be classified under three heads, the first being the study of the nutritive value of proteins, especially vegetable proteins, from the standpoint of maintenance and growth. This work, which began in 1911, already referred to in Chapter IV, and continued to the present time, was conducted in conjunction with the late Thomas B. Osborne of the Connecticut Agricultural Experiment Station. Some forty reports of the results obtained have appeared in various scientific journals, notably the *Journal of Biological Chemistry,* constituting a valuable addition to our knowledge of the nutritive value and dietary deficiencies of the individual proteins, with many observations on the problem of the protein minimum, the relation of the rate of growth to diet, the effects of excessive protein on growth, the effects of mixtures of foodstuffs in unusual proportions, and many other related problems.

The second line of work, also in coöperation with Thomas B. Osborne, had to do with the nutritive value of foods as connected with the presence or absence of vita-

mines, some twenty contributions in which much information of varied character was brought to light. The general scope of this work may be seen from the following titles: *The Influence of Butter-fat on Growth*, 1913; *The Relation of Growth to the Chemical Constituents of the Diet*, 1913; *The Influence of Cod Liver Oil and Some Other Fats on Growth*, 1914; *The Stability of the Growth-promoting Substance in Butter Fat*, 1916; *Milk as a Source of Water-soluble Vitamine*, 1918; *The Vitamines in Green Foods*, 1919; *The Occurrence of Water-soluble Vitamine in Some Common Fruits*, 1920; *Quantitative Aspects of the Rôle of Vitamine B in Nutrition*, 1922; *Egg as a Source of Vitamine B*, 1923; *The Effect of Diet on the Content of Vitamine B in the Liver*, 1923; *The Rôle of Vitamine B in Relation to the Size of Growing Rats*, 1925.

The third series of studies, of somewhat earlier date, bore the general title of *Chemical Studies on Growth* and dealt more especially with the chemical changes taking place in embryonic tissues, ten such studies being reported in the *American Journal of Physiology* during the years 1907 and 1908 by Mendel and several collaborators, notably Charles S. Leavenworth, Tadasu Saiki and Philip H. Mitchell. Mention should also be made of Mendel's studies of *The Paths of Excretion for Inorganic Compounds* conducted with the collaboration of Stanley R. Benedict, Dudley F. Sicher and Henry C. Thacher, during the years 1904-1909, and *The Rate of Elimination of Nitrogen as Influenced by Diet Factors*, a series of studies with Robert C. Lewis, 1913. To give a fully adequate presentation of Mendel's activities in the guidance

of research in physiological chemistry is quite impossible here, but in a later chapter it will be possible to present some of the results of his work in connection with vitamines and growth.

The research activities of Underhill, while he was in the Sheffield Laboratory of Physiological Chemistry, may be classified under the heads of carbohydrate metabolism, the physiological action of tartrates, the physiological action of some protein derivatives, the metabolism of ammonium salts and creatine metabolism, although this by no means covers the extent of his experimental work. In his studies of carbohydrate metabolism the first paper dealt with the *Influence of Hydrazine upon the Organism, with Special Reference to the Blood Sugar Content,* 1911, followed by the *Prevention and Inhibition of Pancreatic Diabetes,* with Morris S. Fine, and the *Production of Glycosuria as a Result of the Intravenous Injection of Witte's Peptone,* in collaboration with Yandell Henderson.

Among other studies in this series the following may be noted: *A Study of the Mechanism of Phlorhizin Diabetes,* 1912; *The Influence of Thyreoparathyroidectomy upon the Sugar Content of the Blood and the Glycogen Content of the Liver,* with Norman R. Blatherwick, 1914; *The Influence of Hydrazine upon the Respiratory Quotient and upon Heat Production,* with John R. Murlin, 1915; *The Rôle of Calcium in the Regulation of Blood Sugar Content,* 1916; *The Control of Acidosis and its Relation to Impaired Sugar Metabolism in Human Diabetes,* 1916; *The Influence of Magnesium Salts upon Blood Sugar Content and upon Epinephrin Hyperglycæmia and*

*Glycosuria*, 1916; *The Relation of the Acid-base Equilibrium of the Body to Carbohydrate Metabolism and its Application in Human Diabetes*, 1917; *The Influence of Acid-forming and base-forming diets upon Blood Sugar Content*, with Louise McDanell, 1917.

The work of Underhill on the behavior of tartrates in the body may be indicated by the following titles: *The Influence of Tartrates upon Phlorhizin Diabetes*, 1912; *The Influence of Sodium Tartrate upon the Elimination of Certain Urinary Constituents during Phlorhizin Diabetes*, 1912; *A Note on the Fate of Tartrates in the Body; Tartrate Nephritis, with Special Reference to Some of the Conditions under Which it May Be Produced; A Study of Renal Secretion during Tartrate Nephritis;* the last three studies being in collaboration with H. Gideon Wells and Samuel Goldschmidt, published in 1913 in the *Journal of Biological Chemistry.*

Among the studies on the metabolism of ammonium salts, the three following may be cited: *The Elimination of Ingested Ammonium Salts in the Dog upon an Adequate Mixed Diet*, 1913; *Elimination of Ingested Ammonium Salts during a Period of Prolonged Inanition*, 1913; *The Utilization of Ammonium Salts with a Non-nitrogenous Diet*, in collaboration with Samuel Goldschmidt, 1913. In the work on the physiological action of some protein derivatives, the three following studies were reported from the laboratory in 1915, being the joint work of Underhill and Byron M. Hendrix; *Are Proteoses Prepared from Zein and Gliadin Physiologically Active?; The Relation of Racemization to the Physiological Action*

*of Proteins and Proteoses; The Physiological Action of Vaughan's "Crude Soluble Poison."*

In the experimental work on creatine metabolism conducted in 1916 the following topics were studied: *Possible Interrelations between Acidosis and Creatine Elimination; The Influence of Alkali upon Creatine Elimination during Inanition; The Influence of Alkali upon the Creatinuria of Phlorhizin Glycosuria; The Relationship of Creatinuria to Carbohydrate Metabolism and Acidosis,* the two last being the joint work of Underhill and Emil J. Baumann. Finally, Underhill's book *The Physiology of the Amino Acids,* published in 1915, gives a good résumé of knowledge of these important chemical compounds.

Arthur H. Smith, a graduate of Ohio State University, 1915, had his training in physiological chemistry in the Sheffield Scientific School at Yale, taking the Ph.D. degree in 1920, after which he served as instructor for four years, being appointed assistant professor of physiological chemistry at Yale in 1924. His more important research work embraces the following topics: *The Effect of Solutions of Certain Salts and Colloids on the Permeability of the Capillary Walls,* with Dr. Mendel, 1920; *The Adjustment of Blood Volume after Injection of Isotonic Solutions of Varied Composition,* with Dr. Mendel, 1920; *The Colorometric Determination of Hemoglobin,* with Barnett Cohen, 1919; *The Relation of Splenectomy to Growth and Appetite in the Rat,* with Lean Ascham, 1922; *Growth on Diets High in Carbohydrate and High in Fat,* with Elizabeth Cary, 1923; A series of six studies on the relationship of *Diet and Tissue Growth,* in collabo-

ration with Theodore S. Moise, 1924-1927; *Growth Experiments on Diets Rich in Fats,* with Harold Levine, 1927; *The Effect of Unilateral Nephrectomy on the Growth of the White Rat,* with Margaret H. Jones, 1927; *Studies on Ketosis in the Rat,* with Harold Levine, 1927; *The Effect of High Protein Diet on the Kidney,* with Theodore S. Moise, 1927.

George R. Cowgill, a graduate of Stanford University, 1916, Ph.D. in physiological chemistry Sheffield Scientific School, Yale, 1921, was made instructor 1921-1925 and assistant professor of physiological chemistry, 1925. His research work has been largely a study of the physiology of vitamines: *A Contribution to the Study of the Relation between Vitamin-B and the Nutrition of the Dog,* 1921; *Vitamin-B and the Secretory Function of Glands,* with Dr. Mendel, 1921; *Is Water-soluble Vitamin Identical with Secretin?,* 1921; *Parenteral Administration of Vitamin B,* 1923; *Vitamin B and the Appetite of the Dog,* with H. J. Deuel, Jr., 1923; *Quantitative Aspects of the Rôle of Vitamin B in Several Species,* with Arthur H. Smith and Howard H. Beard, 1925; *Determination of the Vitamin B Requirement of the Pigeon and its Bearing on the Theory of Vitamin B Function,* with B. H. Klotz, 1927.

Other studies by Cowgill are *The Rôle of the Liver in Pancreatic Secretion,* with N. H. Plummer and H. J. Deuel, Jr., 1924; *The Comparative Action of Pancreatic Secretin when Injected into a Systemic Artery, Systemic Vein and the Portal Circulation,* with H. J. Deuel, Jr., 1924; *Determination of a Formula for the Surface Area of the Dog Together with a Consideration of Formulæ*

*Available for Other Species,* with David L. Drabkin, 1927; *The Relation of Volume, Hydrogen-ion Concentration and Buffer Capacity of the Test Meal to Gastric Contents,* with Welles A. Standish and Alfred T. Shohl, 1927; *Studies on the Effects of Abundant Cereal Intake,* with various collaborators, 1927.

Hubert B. Vickery, appointed lecturer on the chemistry of proteins at Yale in 1924, has been connected with the Connecticut Agricultural Experiment Station since 1922, as research chemist of the Carnegie Institution, and associated with the late Thomas B. Osborne in research work on proteins. A graduate of Dalhousie, B.S., 1915, M.S., 1918, he took the Ph.D. degree in chemistry at Yale in 1922, since which date his activities have been given largely to the study of the chemical constitution of green plants and to the chemistry of proteins.

Thus in the years 1924-1925 he carried on an extensive series of studies on *Some Nitrogenous Constituents of the Juice of the Alfalfa Plant,* dealing especially with *I. The Amide and the Amino Acid Nitrogen; II. The Basic Nitrogen; III. Adenine in Alfalfa,* with Charles S. Leavenworth; *IV. The Betain Fraction; V. The Basic Lead Acetate Precipitate,* with Carl G. Vinson; *VI. Asparagine and Amino Acids of Alfalfa;* all published in the *Journal of Biological Chemistry.* Equally noteworthy were his studies of the hydrolysis of wheat gliadin, *i.e., The Rate of Hydrolysis of Wheat Gliadin,* 1922, and *Mild Acid Hydrolysis of Wheat Gliadin,* 1923, in which the results obtained failed to support the older view advanced by Kühne that hemi and anti groups exist in the protein molecule.

Attention may also be directed to Vickery's work on the *Simpler Nitrogenous Constituents of Yeast*, notably *Choline and Nicotinic Acid,* 1926, and *On the Separation of Histidine and Arginine, the Separation of the Silver Compounds at pH 7.0,* with Charles S. Leavenworth, 1926. In this latter research they were able to accomplish an approximately complete separation of the two bases by bringing the solution containing them, in the presence of an excess of a soluble silver salt, to pH 7.0; a result which led to a recognition of the influence of different hydrogen-ion concentrations on silver precipitations. This influence later, 1928, was made use of advantageously in the analysis of the bases resulting from the hydrolysis of proteins, and in the preparation of pure arginine and histidine in large quantities in typical crystalline form.

Finally, mention should be made of the study of *The Basic Amino Acids of Horse Hemoglobin* carried out with Charles S. Leavenworth, 1928, which gave results "in closest agreement with the assumption that the hemoglobin molecule, weighing 66,800, yields 33 molecules of histidine, 13 of arginine and 37 of lysine."

# CHAPTER XI

*Vitamines—Osborne and Mendel—Work of Hart, McCollum, Steenbock and Humphrey—Work of Elmer V. McCollum and collaborators—Beri-beri, studies by W. P. Chamberlain, E. B. Vedder—Work of Casimir Funk, Robert R. Williams, Atherton Seidell, Walter H. Eddy and collaborators—Xerophthalmia—Carotinoids, studies by Leroy S. Palmer—Origin of Vitamine A—Rickets, studies by Alfred F. Hess, McCollum, E. A. Park and others—Work of John Howland—Action of ultra-violet rays—Scurvy, studies by Hess and Unger, Barnett Cohen and Mendel, H. C. Sherman and collaborators, Ellis, Steenbock and Hart, Cohen and McClugage, R. A. Dutcher—Relation of dietary deficiencies to reproduction, Herbert M. Evans, and K. S. Bishop, Barnett Sure, Mattill and Clayton—Pellagra, experimental work of Goldberger, Vedder, Carl Voegtlin, Underhill and Mendel.*

The discovery of the so-called vitamines with ultimate recognition of the all-important part these substances play in animal nutrition, opened up a new and striking chapter in physiology, development of which has been greatly augmented by the work of American biochemists.

The observations of investigators in many countries working on problems in nutrition had made clear that for some unexplained reason animals fed on a mixture of purified food products, *viz.*, purified proteins, carbohydrates and fats with the needed inorganic salts would not thrive. It was becoming manifest that there were physiological values in natural food products, not indi-

cated by the ordinary methods of chemical analysis and not included in total energy values, that were absolutely essential for growth, maintenance and general well-being. What these physiological values came from, what their nature and chemical characters, remained a mystery. Physiological chemists, however, set out to unravel this mystery and there gradually came to light a multitude of facts, from which eventually it became possible to draw certain general conclusions of great physiological significance. Naturally, at first there was much confusion; many misleading and conflicting results obscured the vision and led to false conclusions, but persistent effort by many workers gradually led to the accumulation of a mass of valuable data from which the truth finally emerged.

In this country Osborne and Mendel in 1911 reported the results of their early feeding experiments with isolated food-substances (Publication 156, Parts I and II, Carnegie Institution of Washington), having in mind especially "the problem of what nitrogenous units are essential for nutrition and whether individual proteins from *different sources and already known to differ in respect to* their chemical make-up vary in their nutritive efficiency." Their results, however, all pointed to one definite conclusion, in harmony with the observations of earlier European workers, *viz.*, that life with normal strength and vigor cannot be maintained on a diet of purified food products, no matter how large the amount nor how varied the components.

Thinking that possibly the mineral ingredients of their artificial diets were in some way inadequate, they resorted to the use of a dried residue of milk after removal of its

fats and proteins, *i.e.*, their so-called "protein-free milk." By using some of this milk residue with isolated proteins, sugar, starch and purified fats, they were enabled to maintain animals such as rats in good physiological condition, with normal growth and power of reproduction. Obviously the "protein-free milk" furnished something essential for adequate nutrition; something not represented by the usual mineral matters and the familiar organic components of ordinary diets. Here was a distinct suggestion of an unknown factor or factors present in the milk residue, essential for growth and physiological well-being.

The next forward step was the discovery that while rats, for example, could be maintained in good condition through two or more generations on a diet of isolated protein, carbohydrate and lard with the protein-free milk, young rats on such a diet after a time failed to grow. This suspension of growth, however, could be prevented by replacing in part the lard by butter fat. In other words, here was evidence that milk fat contains some one or more constituents necessary for growth, with the further suggestion that in milk there may be two essentials, one necessary for maintenance and one for growth. Further, observations of this character tended to show the inadequacy of the old-time methods of determining nutritive values on the basis of analytical data and emphasized the necessity of long-continued feeding experiments capable of indicating differences in true physiological values.

At a somewhat earlier date a group of workers [1] at the Agricultural Experiment Station of Wisconsin was en-

[1] Edwin B. Hart, Elmer V. McCollum, Harry Steenbock and George C. Humphrey. As stated by one of the group, the research in question was planned by Professor Stephen M. Babcock, at that date professor of agricul-

gaged in studying through long-continued feeding experiments the effect of balanced rations from the wheat, oat and corn plants respectively upon the growth and reproduction of cattle. This was a classical experiment with farm animals (commenced in 1906), extending through a long period of time in which it was possible to compare the rate of growth, general appearance, reproduction, lactation, size and vigor of the calves, etc. To summarize in a few words, the corn-fed animals grew normally, continued in a good state of nutrition, gave birth to vigorous calves which in turn developed in normal fashion. On the other hand, the members of the wheat-fed group were poorly nourished, gaunt in appearance and small of girth; their calves were either born dead or died within a few hours. The oat-fed group stood in a position midway between the other two groups.

Here was a striking demonstration that rations, alike so far as chemical analysis would show, having balanced nitrogen content and total fuel value, but from different plants, were radically different in physiological value. With each group, the entire plant was fed, the wheat-fed group, for example, receiving wheat straw, wheat gluten and the entire wheat grain, but obviously something was lacking in two of these diets, something which the corn diet alone supplied.

At the Wisconsin Agricultural Experiment Station much important work bearing on nutrition has been accomplished under the leadership of the workers whose names have already been noted. The following papers

tural chemistry at the University of Wisconsin and chief chemist of the Experiment Station.

may be cited: *Physiological Effect on Growth and Reproduction of Rations Balanced from Restricted Sources,* by E. B. Hart, E. V. McCollum, H. Steenbock and G. C. Humphrey, published in *Research Bulletin 17 of the Wisconsin Station,* 1911; a second paper under the same title published in the *Journal of Agricultural Research,* 1917; *Influence of Rations Restricted to the Oat Plant on Reproduction in Cattle,* by E. B. Hart, H. Steenbock and G. C. Humphrey, published in *Research Bulletin 49 of the Wisconsin Station,* 1920; *The Behavior of Chickens Fed Rations Restricted to the Cereal Grains,* by E. B. Hart, J. G. Halpin and E. V. McCollum, 1917; *Influence of Diet on the Antiscorbutic Potency of Milk,* by E. B. Hart, H. Steenbock and N. R. Ellis, 1921; *Dietary Factors Influencing Calcium Assimilation; The Comparative Influence of Green and Dried Plant Tissue, Cabbage, Orange Juice and Cod-liver Oil on Calcium Metabolism,* by E. B. Hart, H. Steenbock and C. A. Hoppert, 1921; *Studies of Experimental Scurvy, Effect of Heat on the Antiscorbutic Properties of Some Milk Products,* by E. B. Hart, H. Steenbock and D. W. Smith, 1919; *Antineuritic Substances from Egg Yolk,* by H. Steenbock, 1917; *The Fat-soluble Vitamin Content of Roots Together with Some Observations on Their Water-soluble Vitamin Content,* by H. Steenbock and E. G. Gross, 1919; *Thermostability of the Fat-soluble Vitamin in Plant Materials,* by H. Steenbock and P. W. Boutwell, 1920; *The Extractability of the Fat-soluble Vitamin from Carrots, Alfalfa and Yellow Corn by Fat Solvents,* by H. Steenbock and P. W. Boutwell, 1920; *The Fat-soluble Vitamin Content of Green Plant Tissues together with Some Observations*

*on Their Water-soluble Vitamin Content,* by H. Steenbock and E. G. Gross, 1920; *The Fat-soluble Vitamin Content of Peas in Relation to Their Pigmentation,* by H. Steenbock, M. T. Sell and P. W. Boutwell, 1921; *The Fat-soluble Vitamin and Yellow Pigmentation in Animal Fats with Some Observations on its Stability to Saponification,* by H. Steenbock, M. T. Sell and M. V. Buell, 1921; all published in the *Journal of Biological Chemistry.*

The research work carried on at the Agricultural Experiment Station of Wisconsin, of which the above titles are examples, affords another illustration of the important part played by the experiment stations of the country in the development of physiological chemistry, and to Professors Hart and Steenbock much credit is due for the high character of the work in animal nutrition they have accomplished. Edwin Bret Hart, a graduate of the University of Michigan in 1897, a student at Marburg and Heidelberg, associate chemist at the Agricultural Experiment Station of New York, became in 1906 the professor of agricultural chemistry at the University of Wisconsin and chemist of the experiment station there. Harry Steenbock, a graduate of the University of Wisconsin, 1908, a student at Berlin, became associate professor of agricultural chemistry at Wisconsin in 1910 and in 1920 was advanced to the rank of professor of agricultural chemistry. His work on mineral metabolism and on vitamines has been of a high order.

Elmer V. McCollum began his research work in the field of animal nutrition at the University of Wisconsin in 1907, passing on to The Johns Hopkins University in

1917. A graduate of the University of Kansas, 1903, he had his later training in organic and physiological chemistry in the Sheffield Scientific School, taking the Ph.D. degree at Yale in 1906. During the years 1907-1917 as assistant professor, associate professor and professor of agricultural chemistry at Wisconsin he conducted with various co-workers a large number of investigations on the nutritive value of various proteins of vegetable origin and on the distribution and action of vitamines with especial reference to growth.

Among the many papers from McCollum's laboratory the following may be cited: *The Necessity of Certain Lipins in the Diet During Growth*, 1913; *Observations on the Isolation of the Substance in Butter Fat Which Exerts a Stimulating Influence on Growth*, 1914; *Nutrition with Purified Food Substances*, 1915; *The Influence of Certain Vegetable Fats upon Growth*, 1915; *The Nature of the Dietary Deficiencies of Rice*, 1915; *The Essential Factors in the Diet During Growth*, 1915; all with M. Davis and published in the *Journal of Biological Chemistry; The Values of the Proteins of Cereal Grains and of Milk for Growth in the Pig, and the Influence of the Plane of Protein Intake on Growth*, 1914; *The Nature of the Dietary Deficiencies of the Wheat Embryo*, 1916; *The Nature of the Dietary Deficiencies of the Oat Kernel*, 1917; *The Dietary Deficiencies of the White Bean*, 1917; *Dietary Deficiencies of the Maize Kernel*, 1916-1917; *Is Lysin the Limiting Amino-acid in Wheat, Maize or Oats?*, 1916; the last five papers being with N. Simmonds and W. Pitz, published in the *Journal of*

*Biological Chemistry; The Dietary Factors Operating in the Production of Polyneuritis,* with C. Kennedy, 1916.

In 1917, as previously stated, McCollum became the head of the department of chemical hygiene in the School of Hygiene and Public Health at The Johns Hopkins University, since which date he has accomplished a large volume of work bearing on nutrition, especially with reference to vitamines and deficiency diseases. The following studies may be referred to, largely in connection with N. Simmonds and published in the *Journal of Biological Chemistry:* a series of studies under the general title of *A Biological Analysis of Pellagra-producing Diets,* 1917-1919, dealing with such subjects as *The Minimum Requirements of the Two Unidentified Dietary Factors for Maintenance as Contrasted with Growth; Causes of Failure of Mixtures of Seeds to Promote Growth in Young Animals; The Nature of the Dietary Deficiencies of a Diet Derived from Peas, Wheat Flour, and Cotton Seed Oil;* and *Observations on the Faults of Certain Diets Comparable to Those Employed by Man in Pellagrous Districts; The Dietary Properties of the Potato,* 1918; *A Study of the Dietary Essential; Water-soluble B, in Relation to its Solubility and Stability Toward Reagents,* 1918; *A Series of Studies on Supplementary Protein Values in Foods,* with N. Simmonds and H. T. Parsons, 1921, dealing with such matters as the *Supplementary Dietary Relations Between Animal Tissues and Cereal and Legume Seeds;* a series of *Studies on Experimental Rickets,* with N. Simmonds, P. G. Shipley and E. A. Park, 1921, notably *The Production of Rachitis and Similar Diseases in the Rat by Deficient Diets; Effects on Grow-*

*ing Rats of Diets Deficient in Calcium; The Production of Rickets by Diets Low in Phosphorus and Fat-soluble A; Cod Liver Oil as Contrasted with Butter Fat in the Protection Against the Effects of Insufficient Calcium in the Diet; The Antiscorbutic Requirement of the Prairie Dog,* with H. T. Parsons, 1920; *The "Vitamin" Hypothesis and Deficiency Diseases, A Study of Experimental Scurvy,* with W. Pitz, 1917; *The Supplementary Dietary Relationship between the Leaf and Seed as Contrasted with Combinations of Seed with Seed,* with N. Simmonds and W. Pitz, 1917.

While this list of titles affords a very inadequate picture of the accomplishments of McCollum and his co-workers at Baltimore in his earlier years there, it does show clearly the general character of the studies pursued in the attempt to arrive at an understanding of the nature and action of these accessory factors that have to do with growth. Dr. McCollum's results in his animal experimentations bearing on nutrition are correlated with studies of the dietary habits of peoples in different parts of the world in his book entitled *The Newer Knowledge of Nutrition, the Use of Food for the Preservation of Vitality and Health,* the second edition of which appeared in 1922.

From the work of McCollum and his collaborators and that of Osborne and Mendel, supplemented and confirmed by various other investigators both in this country and abroad, it was clearly established that in order to maintain normal nutrition during growth two unidentified substances are necessary: one, soluble in fats and hence accompanying them in their separation from cer-

tain foodstuffs, and the other soluble in water but not in fats. Butter fat stood out particularly as a growth stimulus, while in olive oil, lard and vegetable oils of various kinds the growth factor was absent or present only in small amount, but was found in egg-yolk fat and in cod liver oil. This is the *fat-soluble A* or *vitamine A*, which later experiments by many American workers have shown to be present also in varying amounts in a large number of vegetables such as carrots, chard, lettuce, potatoes, spinach, tomatoes, turnips, squash, peas, cabbage and beans. It is likewise found in corn, wheat embryo and bran, unpolished rice, oats, clover, alfalfa, beef and mutton fat, skimmed milk, orange juice and peel.

The *water-soluble B* or *vitamine B* has likewise been found to be widely distributed. Ordinary meats are apparently poor in this vitamine, but the liver, heart and kidney contain larger amounts as Osborne and Mendel have observed, the proportions being somewhat akin to those found in milk, whole cereals and eggs. Eggs, especially the yolks, appear to contain as much vitamine B as is present in milk solids. While the whole grains of cereals contain a fairly large amount of vitamine B, the highly refined mill products contain much less of the vitamine. Thus Osborne and Mendel found that while 2 to 5 per cent of commercial wheat embryo in an otherwise adequate diet would promote satisfactory growth in the rat, it required 15 to 20 per cent of entire wheat, and at least 50 to 60 per cent of patent flour in the diet to provide sufficient vitamine B for satisfactory growth. In this connection it is to be noted that these same investigators found there were large individual variations in the re-

quirement for vitamine B, doubtless dependent upon the amount stored in the tissues and organs of the body.

The requirements for maintenance and growth are apparently essentially the same. Thus, McCollum states, "By restricting youthful adult animals to diets which were known to be incapable of supporting growth in the young, we have convinced ourselves that there is no important difference in the nutritive requirements of the young and the adult. Any diets which we have studied, which were not satisfactory for the promotion of growth in a young rat, were found to cause some damage to adults which were restricted to them. This might be manifested in early aging, short life, lowered fertility or the deterioration of a family restricted to it through several generations."

Again, it would appear from the experiments of McCollum and Davis that optimum growth, at least, occurs only when the water-soluble and fat-soluble vitamines are both present. Thus, in feeding experiments with young rats, where the dietary deficiencies of polished rice were being studied, they found it impossible to induce growth with polished rice when the latter was supplemented by the addition of purified protein, fats possessing the growth-promoting property, and suitable salt mixtures. If, however, wheat embryo or milk powder, even as small as 2 per cent, were added to the mixture, growth was induced. Even the presence of 20 per cent of butter fat would not induce growth unless the other accessory was present, and on the other hand, the addition of the water-soluble accessory was without effect on growth unless the fat-soluble vitamine was contained in the food mix-

ture. The quantity of water-soluble vitamine required to promote growth is very small. Thus it was found that an alcohol extract of the wheat embryo carrying only 0.6 gram of solid matter and 0.0095 gram of nitrogen, equal to 0.33 per cent of the total nitrogen of the ration, was quite sufficient to insure satisfactory growth.

While much has been learned regarding the occurrence and behavior of these two vitamines, no definite knowledge has as yet been gained as to their exact chemical nature. Benefiting from the facts brought to light by the Dutch chemist Eijkman (1897), by Grijns (1901) and by Hulshoff-Pol (1902) with reference to human beri-beri and the polyneuritis induced in fowls and pigeons by feeding polished rice, notably the fact that both the above diseased conditions could be cured or prevented by the administration of an aqueous extract of the rice polishings, thus implying that beri-beri and the experimental neuritis are due to the lack of one or more antineuritic substances contained in the rice bran and germ, attempts were made to learn something more definite regarding these somewhat intangible substances. As these early studies paved the way for a fuller understanding of antineuritic substances and their relationship to so-called deficiency diseases and also furnished a basis for the belief —which came later—that these antineuritic substances are either identical with or closely akin to the accessory food factors now known as vitamines A and B, brief mention may be made of their findings.

With the occupation of the Philippines by the United States, various members of the United States Army Medical Commission for the investigation of tropical diseases

carried on a series of studies dealing with beri-beri, in which many interesting facts were brought to light. The following papers may be cited: *Eradication of Beri-beri from the Philippine Native Scouts by Means of a Simple Change in Their Dietary,* by W. P. Chamberlain, 1911; *A Contribution to the Etiology of Beri-beri,* by W. P. Chamberlain and E. B. Vedder, two papers, 1911, and a third paper with R. R. Williams, 1912; *A Study of the Influence of Rice Diet and of Inanition on the Production of Multiple Neuritis in Fowls and the Bearing Thereof on the Etiology of Beri-beri,* by W. P. Chamberlain, H. D. Bloombergh and E. D. Kilbourne, 1911; *The Cure of Infantile Beri-beri by the Administration to the Infant of an Extract of Rice Polishings and the Bearings Thereof on the Etiology of Beri-beri,* by W. P. Chamberlain and E. B. Vedder, 1912; published mainly in the *Philippine Journal of Science.*

The work accomplished by these American investigators in the Philippines did much to facilitate the studies of later workers. Thus, they found that the substance responsible for the cure and prevention of beri-beri and of experimental polyneuritis was soluble in water and alcohol, insoluble in ether, easily dialyzable, fairly thermostable, contained nitrogen, but was not an amino-acid of known constitution, nor an alkaloid, neither was it a phosphorus compound, but might prove to be a nitrogenous base.

Mention must next be made of the work of a biochemist who does not belong to America, but who for a time was connected with the Medical College of Cornell University, in cancer research, 1915-1916, and as associate

biochemist in the College of Physicians and Surgeons of Columbia University, 1921-1923, since which date he has been head of the department of biochemistry in the School of Hygiene at Warsaw. Casimir Funk, a student and worker in several countries, notably in Germany and in England, was the first to claim the isolation of the chemical substance responsible for the antineuritic action of rice polishings.

Using large volumes of extract of these polishings Funk obtained by careful chemical treatment various fractions which were tested for their antineuritic power upon polyneuritic fowls. By frequent repetition of this fractioning and testing he obtained eventually a very small quantity of a crystalline substance which was found to be possessed of curative power in high degree. He likewise detected this same substance in brewer's yeast where it appeared relatively more abundant and by appropriate method of treatment he was able to isolate it in crystalline form. It is to be noted that the active substance was completely precipitated from aqueous solution by phosphotungstic acid and on decomposition of this precipitate with barium hydroxide the curative substance was obtained free from protein, carbohydrate and phosphorus. It appeared to be a nitrogenous base, possibly a member of the pyrimidine group analogous to adenine.

Since this substance was essential to life and its chemical structure appeared to be that of an amine Funk gave to it the name vita-amine or vitamine, hence the origin of this now widely used term. Unfortunately the work of later years has not given support to all of Funk's views regarding the chemical nature of this substance and con-

sequently there has been a tendency to employ a different terminology, but since the substances of this class are still mysterious from a chemical standpoint, all that is really necessary is a distinguishing term.

Of Funk's work that appeared in America, the following may be noted, published in the *Journal of Biological Chemistry: On the Probable Nature of the Substances Promoting Growth in Young Animals,* with A. B. Macallum, 1915; *The Study of Certain Dietary Conditions Bearing on the Problem of Growth in Rats,* 1916; *The Comparative Value of Lard and Butter Fat in Growth,* with A. B. Macallum, 1916; *The Action of Yeast Fractions on the Growth of Rats,* 1916; *A Test for Antiberiberi Vitamin and its Practical Application,* with H. E. Dublin, 1920; *The Vitamins of Yeast and Their Rôle in Animal Nutrition,* with H. E. Dublin, 1921, published in the *Proceedings of the Society of Experimental Biology.*

Robert R. Williams, for a time research chemist in the Bureau of Science in the Philippines, 1908-1915, later in the United States Department of Agriculture and since 1925 research associate in Teachers College, Columbia University, made many attempts to isolate the active principle from rice polishings, using modifications of Funk's method, but he succeeded in obtaining only very small yields of the crystalline substance. This, however, had curative power in the treatment of human beri-beri and when administered to polyneuritic fowls. Abandoning the attempt to separate the active vitamine in quantity, Williams devoted his efforts toward a study of synthetical compounds which might have curative power, hoping thereby to obtain some light on the chemical nature of the

vitamine. As the work of Funk and others had shown that the vitamine fraction from rice polishings, the phosphotungstic acid precipitate, on being further fractioned yielded various nitrogenous substances, among which nicotinic acid was conspicuous, it appeared possible that the pyridine ring might be the nucleus of the vitamine.

Studying the antineuritic power of various synthetic hydroxypyridines Williams found that α-hydroxypyridine, 2, 4, 6-trihydroxypyridine, and 2, 3, 4-trihydroxypyridine were curative, when first prepared, but that gradually their curative power disappeared. This loss of power he attributed to isomerization, this view being strengthened by the fact that in studying the isomeric forms and curative power of α-hydroxypyridine it was observed that of the two crystalline forms only one was active. These and other like results obtained with β- and γ-hydroxypyridine led Williams to make the following statement: "The antineuritic properties of these substances suggest that an isomerism is at least partially responsible for the instability of the vitamin in food stuffs and that the antineuritic property may be inherent in the potentiality of this type of isomerism."

The following papers contain the results of Williams studies: *The Chemical Nature of the Vitamins, Antineuritic Properties of the Hydroxypyridines*, 1916; *The Structure of the Curative Modifications of the Hydroxypyridines*, 1917; *Isomerism in Natural Antineuritic Substances*, with A. Seidell, 1916; all published in the *Journal of Biological Chemistry; The Chemistry of the Vitamins*, 1916; *Experimental Treatment of Human Beri-beri with Constituents of Rice Polishings*, with N. M. Saleeb

915; both published in the *Philippine Journal of Science; Vitamins from the Standpoint of Structural Chemistry,* 1921; published in the *Journal of Industrial and Engineering Chemistry.*

Atherton Seidell of the United States Public Health Service found (1916) that hydrous aluminum silicate (a form of fullers' earth) had the power of adsorbing the vitamine from autolyzed yeast solutions, and taking advantage of this he was able to prepare specimens of vitamine having curative effect on completely paralyzed pigeons. Later, 1921, he reported the preparation of an amorphous silver compound from activated fullers' earth which when administered to pigeons prevented the development of polyneuritic symptoms. While this work was promising in its possibilities, it has not as yet led to any definite result regarding the chemical nature of the vitamine.

Seidell's more important contributions are the following: *Vitamins and Nutritional Diseases, A Stable Form of Vitamin, Efficient in the Prevention and Cure of Certain Nutritional Deficiency Diseases,* 1916; *Preliminary Note on a Stable Silver Vitamin Compound Obtained from Brewers' Yeast,* 1921, both published in United States Public Health Reports, Nos. 31 and 36; *The Vitamin Content of Brewers' Yeast,* 1917, *Journal of Biological Chemistry; The Chemistry of Vitamins,* 1921; *Experiments on the Isolation of the Antineuritic Vitamin,* 1921; both published in the *Journal of Industrial and Engineering Chemistry.*

Osborne and Wakeman, 1919, reported under the title *Extraction and Concentration of the Water-soluble Vita-*

*mine from Brewers' Yeast* the results of their efforts to separate the vitamine from the crude material of the yeast. The method employed was essentially a series of fractional precipitations with alcohol, by which much of the inert material was removed, the vitamine fraction containing practically the entire quantity of vitamine originally present in the yeast. While the final fraction was rich in vitamine, with a potency ten times greater than that of the original material, it contained a variety of substances which gave no clue to the chemical nature of the vitamine. P. A. Levene and others have employed silica gel for adsorption of the vitamine present in yeast, but with no great advance in knowledge of the substance.

In 1924 there appeared a paper from the laboratory of physiological chemistry at Teachers College, Columbia University, by Walter H. Eddy, Ralph W. Kerr and R. R. Williams, entitled *The Isolation from Autolyzed Yeast of a Crystalline Substance Melting at 223°, Having the Properties of a Bios* (*J. Am. Chem. Soc.*, 46:2846). The separation of this substance involved selective adsorption or precipitation by ferric oxide hydrosol and recovery by removal of the iron with barium hydroxide. It was crystalline, belonging to the orthorhombic system, and had a composition in harmony with the formula $C_5H_{11}NO_3$, with a molecular weight of approximately 133. The authors suggested that its structure might be that of a heterocyclic nitrogen-carbon ring with a carboxyl group attached. This substance was possessed o marked stimulating action on the growth of yeast eve when present only to the extent of 0.005 milligram pe cubic centimeter. It did not, however, show any ant

neuritic power. P. A. Levene and Muhlfeld, 1923, emphasized their belief that the antineuritic and growth-promoting principles are not identical.

J. J. Willaman and Aksel G. Olsen of the Division of Agricultural Biochemistry, University of Minnesota, under the title *The Bios Requirement of Bakers' Yeast,* 1923, reported as a result of their studies that while bios is of the nature of a vitamine, and that the natural growth of yeast is impossible without bios, yet the latter is not identical with water-soluble B vitamine. However, under the general term vitamine B are usually included the antineuritic vitamine, the lack of which leads to polyneuritis in birds and fowls, to beri-beri in man and to like pathological conditions in other mammals; also the water-soluble vitamine which promotes growth and which may be identical with the antineuritic vitamine and lastly bios, together with other growth-promoting substances formed by bacteria in soil as an aid to the growth of green plants. Another conception is that the water-soluble vitamine B is made up of two distinct bodies: one, the antineuritic or anti-beri-beri vitamine, and the other, the pellagra-preventing vitamine. Until more definite knowledge of the chemical nature of the vitamines is at hand no sharp differentiation is possible.

Since there is no clear understanding of the chemical structure of the vitamines and no chemical method for their detection or measurement, progress of knowledge has depended almost entirely upon studies of the nutritional needs of animals as revealed by feeding experiments, noting particularly the effect of various dietaries on maintenance, growth, reproduction, etc.; also the ef-

fect on diseased animals suffering from certain dietary deficiencies, as in beri-beri, scurvy, edema, opthalmia, pellagra, rickets, polyneuritis of birds, etc. The importance of these accessory factors in normal dietaries, *i.e.*, the vitamines, has been demonstrated with equal clearness by observations on growth, maintenance of normality and other conditions, and by observations on the curative effects in deficiency diseases. Such studies have likewise led to the discovery of additional vitamines endowed with specific functions.

While Casimir Funk was unquestionably the first to emphasize the relationship between certain diseases and faulty diet, that is a lack of vitamines in the food, he had up to 1910 succeeded in inducing only one deficiency disease, namely, beri-beri or polyneuritis, by feeding animals solely on polished rice. After this date, as previously mentioned, the experimental work of a large number of investigators, notably Osborne and Mendel, McCollum and his collaborators, and Hart and Steenbock, has thrown a flood of light on food accessories both in relation to growth and to deficiency diseases, work which has been confirmed and enlarged in many different laboratories. Such studies have also brought out very clearly the fact, that since proteins are not all built alike, varying greatly in the character and amount of their constituent amino-acids, growth is dependent largely upon the character of the protein fed, *i.e.*, whether it contains all the amino-acids needed for the formation of the body protein and in proper proportion.

Further, it became equally clear, from experimental evidence, that the necessary inorganic constituents may not

be present in certain foods in amounts sufficient to meet the requirements of young animals during the period of growth. Thus, in feeding experiments with wheat, already referred to, McCollum and Davis, 1915, found that in the wheat kernel there are three dietary factors of poor quality and only when the wheat kernel was supplemented with protein, a salt mixture and a growth-promoting fat (butter fat) could animals be maintained, with normal growth, healthy young and long period of life.

It thus becomes obvious that in studying the effects of a given dietary attention must be directed to the amino-acid content, and the mineral supply as well as to the vitamine content. With these indispensable variables in the diet, with perhaps other factors not yet recognized, it is manifest that great care must be exercised in the adjustment of experimental conditions.

Manifestation of the presence or absence of vitamines takes on many forms, some very specific and characteristic. Thus, in 1913 Osborne and Mendel reported that their experimental animals (rats) when fed on a diet free from fat tended to develop a characteristic eye disease (ophthalmia, xerophthalmia, keratoconjunctivitis) which was quickly cured by the addition of butter fat or cod liver oil to the diet. Naturally, they concluded it was the absence of the fat-soluble vitamine A that caused the appearance of this disease. Cure of this abnormal condition by treatment with the usual antiseptic agents could not be accomplished but "a single drop of good cod liver oil each day or a few milligrams of a suitable extract of green leaves will suffice to effect such cures in rats." That the absence of vitamine A was solely responsible for the

appearance of the disease was indicated by the fact that of five hundred rats living on deficient diets during a period of one year only those having a diet deficient in vitamine A showed any eye symptoms. Of 136 rats living on a diet lacking vitamine A, 69 developed this eye disease (Osborne and Mendel).

Observations along these same lines showed that other species of animals have the same susceptibility to eye trouble when living on dietaries poor in vitamine A. Thus, V. E. Nelson and A. R. Lamb found the same diseased conditions in rabbits on a diet deficient in vitamine A, reported in the *American Journal of Physiology,* 1920, under the title *The Effect of Vitamin Deficiency on Various Species of Animals, 1, The Production of Xerophthalmia in the Rabbit.* With dogs, similar results have been reported by Steenbock, Nelson and Hart, 1921.

It has been suggested by several investigators that xerophthalmia as found in man may be one of the results manifested by lack of the fat-soluble vitamine. Experimental animals deprived of vitamine A are also liable to suffer from other disturbances, such as weakness of various tissues and organs, diarrhea, diminished appetite, renal calculi of a phosphatic nature, cutaneous malnutrition, especially noticeable in rats (Osborne and Mendel), and an increased susceptibility to infections of the respiratory system (McCollum, Steenbock and others). From which it is plain that vitamine A, in addition to being essential for growth, is likewise a determining factor in the maintenance of a normally healthy condition.

Remembering that vitamine A as found in the animal body is largely associated with lipoid or fatty substances,

as in butter fat, cod liver oil and egg yolk fats, in which it is freely soluble, it was assumed at one time that the vitamine might be a fat or lipoid, but all the experimental evidence so far obtained has failed to furnish any evidence of its identity with any known lipoid, fatty acid or glyceride. Of special interest was the fancied relationship between the yellow pigment of fats and vegetable tissues and the content of vitamine A. Thus observations by Steenbock and his co-workers, 1919, showed that carrots and yellow sweet potatoes were relatively richer in this vitamine than potatoes, sugar beets, mangels, parsnips, etc. Again yellow maize was found to be quite efficient as a source of the fat-soluble vitamine while white maize was distinctly deficient in this food accessory. Red maize free from yellow pigment was also lacking in the vitamine, but when the maize contained yellow pigment good results were obtained in feeding experiments.

Osborne and Mendel, 1919, called attention to the richness of spinach, clover leaves and alfalfa leaves in fat-soluble A, while cabbage was comparatively deficient in this vitamine and lettuce leaves appeared to be poorer in the vitamine than many other leaves studied. While the green leaves of plants are the most important source of vitamine A in the vegetable kingdom, it is to be remembered that yellow pigment is also abundant in such leaves, although masked by the green. Green peas were found by Steenbock to be rich in yellow pigment and to contain more vitamine A than yellow peas not so highly pigmented. Steenbock, 1919, especially among American workers held the belief that the fat-soluble vitamine A was one of the plant yellow pigments, this view being

ased on a large number of data, all tending to emphasize he close relationship between yellow pigmentation and itamine content.

On this subject much work has been done by Leroy S. Palmer, professor of agricultural biochemistry at the University of Minnesota since 1922 and prior to that date, 1913-1919, assistant professor of dairy chemistry at the University of Missouri. His more important contributions may be cited: *The Physiological Relation of Plant Carotinoids to the Carotinoids of the Cow, Horse, Sheep, Goat, Pig and Hen,* 1916; *Carotinoids as Fat-soluble Vitamin,* 1919; *Carotin, the Principal Natural Yellow Pigment of Milk Fats, Its Relation to Plant Carotin, and the Carotin of the Body Fat, Corpus Luteum and Blood Serum,* with C. H. Eckles, 1914; *The Relation of Plant Carotinoids to Growth, Fecundity and Reproduction of Fowls,* with H. L. Kempster, 1919; *The Relation of Plant Carotinoids to Growth and Reproduction of Albino Rats,* with C. Kennedy and H. L. Kempster, 1921, all published in the *Journal of Biological Chemistry.*

From the results of the many experiments reported by Palmer and his collaborators it became clear that the so-called yellow pigment theory of the fat-soluble vitamine was not tenable. Thus chicks were hatched and raised on a mixture of white maize, white maize bran, skim milk, etc., entirely free from carotinoids or yellow pigment, the hens beginning to lay when six months old. Many of these eggs were incubated, viable chicks being hatched normal in every detail except for the absence of yellow pigment on legs, beaks and other parts usually pigmented. Again, they found it quite possible to secure

normal growth and reproduction in albino rats fed on foods nearly or quite free from carotinoid pigments, the necessary fat-soluble A being supplied by colorless ewes' milk fat, or colorless egg yolk, while vitamine B was furnished by extract of the wheat germ or bakers' yeast.

Regarding the origin of vitamine A, observers agree that the evidence at present available indicates that the animal body cannot synthesize or manufacture it and that consequently it must come entirely from the food, *viz.*, green leaves, in lesser degree from some roots, and the embryos of seeds, while milk, eggs and butter are likewise rich in this vitamine. Obviously the primary source of the vitamine is in plants, from which that found in animal products must have been derived. Osborne and Mendel and also McCollum have pointed out that hay, clover, alfalfa and other grasses provide an abundant supply of vitamine A for cattle, even when fed dry, this food accessory passing into the milk of the cows.

It is plain that the amount and character of the food fed determines the store of vitamine A in the animal body. Thus, H. C. Sherman and his collaborators, 1920, found that mature rats fed on diets lacking vitamine A, but otherwise satisfactory, would survive for periods which varied greatly according to the nature of the previous dietary and its richness in vitamine A. As Sherman [2] has expressed it, "The vitamine A which the body stores when well fed probably constitutes a very important resource which can be drawn upon to enable the organism to meet either the normal increased demands of reproduction and

[2] Sherman, H. C., and Smith, S. L., "The Vitamins," American Chemical Society Monograph Series, 1922.

lactation, or emergencies such as subsequent dietary deprivations." The normal storage of vitamine A in the animal body under adequate dietary conditions must be an important aid in the maintenance of good health and resistance to disease as various observers have pointed out. In this connection it is to be noted that the storage capacity of the animal body for vitamine A is much greater than that for the other vitamines.

In 1919 an English investigator, E. Mellanby, reported results with young puppies which indicated that such fats as contain vitamine A, notably cod liver oil and butter fat, protected the young animals from rickets. Vegetable fats on the other hand showed no such power. From these experiments arose the view that the fat-soluble vitamine A, or perhaps some other substance having a like distribution, is a specific anti-rachitic body, designated by some authorities as vitamine D.

That something more than the vitamine content of the food is involved in rickets was, however, indicated by the experimental work of H. C. Sherman of Columbia University and A. M. Pappenheimer, *Experimental Rickets in Rats, I, A Diet Producing Rickets in White Rats, and its Prevention by the Addition of an Inorganic Salt,* published in the *Journal of Experimental Medicine,* 1921. In fact, their results appeared to show "that rickets may be caused or prevented by changes in the mineral elements of the food without any alteration of either the protein or vitamin components of the diet." Sherman considered that the inorganic constituents of the diet were more directly connected with the disease than vitamine

A, especially lack of phosphate and the ratio between calcium and phosphorus.

Alfred F. Hess, clinical professor of pediatrics at University and Bellevue Hospital Medical College, with various collaborators has carried on numerous studies bearing on infantile rickets and on experimental rickets in rats. In 1920, with L. J. Unger (*Journal of the American Medical Association*) he reported the results of feeding experiments on infants, with a diet very low in the fat-soluble vitamine covering periods of from five to nine months in which satisfactory growth was obtained without the appearance of rickets. Again in 1921, with G. F. McCann and A. M. Pappenheimer, he published in the *Journal of Biological Chemistry* experiments with rats under the title *Experimental Rickets in Rats, II, The Failure of Rats to Develop Rickets on a Diet Low in Vitamine A*, where again the absence of appreciable amounts of the fat-soluble vitamine did not lead to the development of rickets. Hence, there seemed justification for the view that vitamine A was at most of minor importance in the protection from rickets. Possibly, however, there is truth in the criticism made by several workers in this field that the diet employed was not as deficient in vitamine A as was believed at the time.

One of the striking facts connected with rickets is the curative or preventive action of cod liver oil. As Osborne and Mendel found, this oil is rich in vitamine A, but according to McCollum and others there is another organic substance present, designated as X, distinct from the fat-soluble vitamine, which has the power of causing calcium salts to be deposited in the bones in rickets. Thus, as Mc-

llum has observed, the addition of two per cent of cod er oil to the diet of rats—rich in calcium and poor in .osphorus and vitamine A, with an unfavorable ratio r the deposition of calcium salts in the growing bones -causes within a few days the beginning of a calcification hich is quite marked. Butter fat, on the other hand, even › the extent of fifty per cent of the diet, was found to e comparatively ineffective in stimulating the deposition f calcium phosphate under the above conditions.

Again, as McCollum has stated, "the remarkable effect f the administration of cod liver oil to animals which are leprived of sufficient calcium, in protecting them from he detrimental effects of such deficiency—a protection which is not afforded by four or five times the amount of butter fat necessary to entirely meet the needs of the growing rat for fat-soluble A, when its diet is satisfactorily constituted with respect to calcium and phosphate, makes it imperative that we accept the view that there is an organic factor which exerts an anti-rachitic effect and is concerned in the normal nutrition of the bones."

Much light on the etiology of rickets, on the protective action of the organic factor concerned in the causation of the disease and the effect of sunlight on experimental rickets, has been shed by the combined work of McCollum, N. Simmonds, P. G. Shipley and E. A. Park, at The Johns Hopkins University, under the general title of *Studies on Experimental Rickets,* already cited. Among the many results reported by these investigators the following may be noted: lack of vitamine A in the diet of young rats containing proper amounts of calcium and phosphate leads to pathological changes in the bone, the same changes

occurring when there is a deficiency of the so-called anti-rachitic factor in the diet; excessive amounts of calcium in the diet with relatively small amounts of phosphate and vitamine A cause rickets in young animals due, as these observers believed, not to variations in the absolute amounts of the salts present, but rather to the ratio between the concentration of calcium and of phosphorus in the diet; the protective action of the organic factor which plays a part in the development of rickets manifests itself when cod liver oil is added to the diet of animals so prepared that there is an unfavorable ratio between the calcium and phosphorus, the addition of the accessory substance causing at once a deposition of calcium.

According to the views of McCollum, 1922, rickets is not a deficiency disease in the sense that beri-beri and dietary ophthalmia are—due to starvation for one or more organic substances—but is due to a disturbance in the ratios of at least three dietary factors, *viz.*, calcium, phosphorus and the anti-rachitic substance, so abundant in cod liver oil. The striking effects of cod liver oil in inducing deposits of calcium salts in the bones in rickets were clearly shown by H. Steenbock, J. H. Jones and E. B. Hart in experiments reported in 1923, *Stability of Vitamine in Cod Liver Oil*, in the *Journal of Biological Chemistry.*

Using puppies as subjects they made use of a ration composed of white corn meal, oat meal, casein, sodium chloride, calcium phosphate and skimmed milk, a ration highly nutritive but lacking in the substance or substances characteristic of cod liver oil. On this diet the young animals failed to grow, muscular spasms developed, the

calcium and phosphorus content of the blood serum dropped much below normal and the bones were poorly calcified. When, however, a few cubic centimeters of cod liver oil were introduced into the daily ration the above abnormalities failed to appear; there was normal growth, normal content of calcium and phosphorus in the blood serum and normal distribution of mineral matter in the bones.

John Howland (professor of pediatrics at The Johns Hopkins University, 1912 until his death in 1926) in collaboration with B. Kramer, published in 1920-1921 the results of studies of the blood salts in rickets, *Calcium and Phosphorus in the Serum in Relation to Rickets, American Journal of Diseases of Children,* 1921, and *Journal of Biological Chemistry,* 1920, in which it was found that while in normal children the blood serum contained 10-11 milligrams of calcium per 100 cc., in tetany complicated with rickets the amount was reduced to half the above figures. If, however, the rickets was not complicated by tetany the calcium content remained normal. The phosphate content of the blood serum, on the other hand, frequently fell to half the normal amount. Thus, the inorganic phosphorus in the normal blood of children Howland and Kramer found to be about 5 milligrams per 100 cc. while in children suffering from rickets the amount might drop as low as 0.8 milligram per 100 cc. If cod liver oil was given to such rachitic children the phosphorus content of the blood serum rose to the normal or even above.

Another agency potent in preventing the development of rickets and equally potent in the cure of the disease

is radiant energy, ultra-violet rays and direct sunlight. This factor has special significance since it may aid in bringing about an understanding of the true nature of these protective functions associated with the vitamines in general. While the first demonstration of the value of ultra-violet rays in the treatment of rickets was made in Germany, 1919, American investigators have contributed much, notably Alfred F. Hess and various collaborators.

The following papers by Hess may be cited: *The Cure of Infantile Rickets by Artificial Light and by Sunlight*, with L. J. Unger, 1921; *The Prevention of Rickets in Rats by Exposure to Sunlight*, with L. J. Unger and A. M. Pappenheimer, 1921; *Antirachitic Properties Imparted to Inert Fluids and to Green Vegetables by Ultra-violet Irradiation*, with Mildred Weinstock, 1924; *The Antirachitic Value of Irradiated Phytosterol and Cholesterol*, with Mildred Weinstock and Dorothy Helmar, 1925, the two latter published in the *Journal of Biological Chemistry*.

Among the many facts established by the work of Hess and his associates the following have special significance: Cotton-seed oil and linseed oil, both of which are free from any known vitamine and are without action as antirachitic agents, can be rendered specifically active by irradiation with the mercury vapor lamp, the oils after irradiation being able to protect rats from rickets even when only 0.1 cc. daily is added to the rickets-producing dietary. Likewise, various foods which are inactive or which in their natural state have no antirachitic power can be rendered active in this respect by means of ultra-

violet irradiation. Similar results have been obtained by Steenbock and his collaborators.

On the other hand, chlorophyl, hemoglobin, red blood cells, cream, the phosphatide of egg yolk and glycerol were not activated by irradiation. Green lettuce leaves from the market were found to be valueless in preventing rickets, but after irradiation they became antirachitic. Wheat grown in the dark was found to have no antirachitic potency, but when grown in the light and irradiated with the mercury vapor lamp it conferred protection on rats. Hence, it follows, as Hess states, that an antirachitic factor was produced both *in vitro* and in the growing plant. Both wheat and vegetable oils which had been activated by ultra-violet irradiation were found to retain their antirachitic potency for a considerable period; wheat for several weeks and the vegetable oils for at least six months.

Whatever the reaction that takes place when an antirachitic factor is produced by irradiation it would appear from the observations of Hess and his co-workers that oxygen plays no part in the process since the reaction will take place in an atmosphere of nitrogen. Further, it was found by fractionization that the active principle is present only in the non-saponifiable moiety of the irradiated oil, mainly phytosterol. While phytosterol and cholesterol have in themselves no protective action against rickets, when irradiated they both show antirachitic power, and irradiated cholesterol was found to be effective in preventing the rickets caused by a diet low in phosphorus or low in calcium. Experiments by Hess and Windaus, 1926, brought out the interesting fact that *purified* choles-

terol fails at times after irradiation to manifest any curative or protective action on rickets, due probably to the removal of some admixture or impurity such as ergosterol.

Ergosterol, which is an optically active sterol having three double bonds and a hydroxyl radicle, after irradiation proved to be very effective with rachitic rats, doses of 0.003 milligram daily being sufficient to induce healing of the bones. It has been suggested (Windaus) that ergosterol is a pro-vitamine, which under the influence of the ultra-violet rays is converted into the antirachitic vitamine or factor, through some change in the configuration of the molecule.

Powers, Park, Shipley, McCollum and Simmonds, in studying the action of sunlight as a preventive of rickets in rats, 1922, used two groups of animals fed on the same diet—a diet which previous experience had shown would induce rickets in a few weeks—one group being kept in a room with very little illumination, while the other group was exposed to more or less direct sunlight for a period of two months with an average daily exposure of four hours. The control animals all showed severe rickets at the end of the period, while the animals exposed to sunlight were entirely free from the disease. It was also noted "that the good effect of the illumination was not limited to the bones, but had a profound influence on all the cells of the body." (McCollum.) Mention must also be made of some experiments by B. Kramer, 1925, on the irradiation of cow's milk, in which he found that 10 to 20 minutes' exposure caused the milk to manifest antirachitic power sufficient to cure rickets in young children.

In the words of Professor E. A. Park,[3] "Rickets is a disturbance in the metabolism of the growing organism of such nature that the salt equilibrium, in particular as regards the calcium and phosphorus, in the circulating fluids is disturbed, and lime salts no longer deposit in the bones. Lime salts may not deposit because the ionized calcium in the blood is low, or because the ionized phosphate is low, or because both are low. . . . Two factors exist, the one in radiant energy, the other in an unknown form in certain foods, either of which is capable of preventing rickets from developing or from continuing, if already established. . . . Only when the organism is deprived of the influences of radiant energy and of X can rickets develop. . . . Increased knowledge has indicated that the rôle played by radiant energy and X in the maintenance of the normal salt metabolism is of the utmost importance, and that the organism is dependent on the energy of the sun's rays or of their equivalent in the food to an extent little appreciated." Much, however, remains to be learned regarding the antirachitic vitamine.

Another deficiency disease, scurvy, long known as associated with narrow and restricted dietaries and prevented or cured by the use of vegetables and fresh fruits, became the subject of laboratory experiments when the vitamine hypothesis was established. Early investigations, 1912, by Europeans, notably Holst and Frölich of Christiania, were directed largely to the testing of a great variety of foods for antiscorbutic properties, from which it was definitely shown that such foods as potatoes, raw cabbage, carrots, onions, apples, endive, lettuce and dandelion leaves would

[3] *Physiol. Rev.*, 3: (1923).

prevent the development of scurvy in guinea pigs when added to a diet which without such addition caused the disease.

In this country, the work of A. F. Hess has given much information regarding the disease in infants and children, while the experimental work of Hess and Unger has contributed largely to an understanding of the scurvy of guinea pigs. The following papers by Hess may be cited: *Infantile Scurvy,* a series of studies dealing with various aspects of the subject, such as *Its Influence on Growth,* 1916; *The Therapeutic Value of Yeast and of Wheat Embryo,* 1917, both published in the *American Journal of Diseases of Children; Chemistry of the Blood in Scurvy,* with J. Killian, 1918, *Proceedings Society of Experimental Biology and Medicine; The Antiscorbutic Vitamin,* 1921, *Journal of Industrial and Engineering Chemistry.*

Of studies by Hess and L. J. Unger, the following may be noted: *The Scurvy of Guinea-pigs, I, The Experimental Dietary, II, Experiments on the Effect of the Addition of Fruits and Vegetables to the Dietary,* 1918, *Journal of Biological Chemistry; The Effect of Age, Heat and Reaction on Antiscorbutic Foods,* 1919; *Factors Affecting the Antiscorbutic Value of Foods,* 1919, *American Journal of Diseases of Children; The Destructive Effect of Oxidation on Antiscorbutic Vitamin,* 1921, *Proceedings Society of Experimental Biology and Medicine; Relation of Fodder to the Antiscorbutic Potency and Salt Content of Milk,* with A. C. Supplee, 1920, *Journal of Biological Chemistry.*

In 1918 Barnett Cohen and L. B. Mendel reported in

the *Journal of Biological Chemistry* the results of a comprehensive study of scurvy under the title *Experimental Scurvy of the Guinea-pig in Relation to the Diet.* Employing a ariety of diets, in some cases limited to a single grain such as oats or barley, in other cases a mixture of grains, supplemented by such additions as were needed to maintain normal nutrition, it was found that on an exclusive diet of cereal grains scurvy was readily induced, but if the grains were germinated before being used the onset of scurvy was delayed or even prevented. On a diet of oats and milk, the antiscorbutic factor being provided solely by the milk, it was noted that the addition of 85-135 cc. of milk per day was required for the complete protection of a guinea pig, while 50 cc. of milk delayed the appearance of the disease for two months and a half.

With a diet of soy bean flour properly cooked and with the addition of small amounts of sodium chloride and calcium lactate, yeast in sufficient quantity to furnish the necessary vitamine B, and Jersey milk with its contained fat to provide the required vitamine A, *i.e.,* everything needed for good nutrition except the antiscorbutic vitamine, young guinea pigs showed for a time satisfactory growth but after the tenth day symptoms of scurvy begar to manifest themselves in the shape of tenderness of th joints followed by a marked swelling of the wrists, ankle and knees. This swollen condition of the joints speedil grew worse and after a time the joints frequently becam swollen to two or three times their normal size, whi at the same time there was a loss of appetite and a d cline of body weight. If not too long delayed, the additi to the diet of foods containing a requisite amount of vi

mine C led to recovery. Of various antiscorbutic foods, fresh cabbage and cabbage dried at about 75°C., fresh carrots, orange juice, germinated oats and barley were all found to be effective in preventing scurvy.

The investigations of Hess and his collaborators, of Mendel and co-workers, as well as the studies of many other American investigators, to be referred to later, have helped make clear that scurvy is not only a deficiency disease but that it is due to the lack of a specific vitamine, now known as vitamine C. Like vitamine B, the antiscorbutic vitamine was found to be readily soluble in water and in alcohol, but was not adsorbed by fuller's earth. Vitamine C is apparently less stable than vitamine B, being more readily destroyed, *i.e.*, its antiscorbutic potency diminished, both by oxidation and by heating. It has been suggested that the vitamine may be an enzyme or an enzyme-like substance, but its ready solubility in alcohol and its behavior towards heat are opposed to this view. As H. C. Sherman has expressed it, "The low temperature coefficient of the heat destruction of vitamin C, its solubility in alcohol, and its diffusibility, all count strongly against the view that the vitamin may be an enzyme-like substance."

Experiments by V. K. La Mer, H. L. Campbell and H. C. Sherman—*The Effect of Temperature and of Hydrogen-ion Concentration upon the Rate of Destruction of Antiscorbutic Vitamin,* 1921, *Proceedings Society of Experimental Biology and Medicine;* also *Journal American Chemical Society,* 1922—using filtered tomato juice showed that while the vitamine therein was gradually destroyed by heat, the temperature coefficient of the rate

of destruction was considerably lower than is the case with ordinary chemical reactions, where a rise of 10° C. generally doubles the rate of the reaction. With tomato juice, however, it was found that a rise of 10 degrees in temperature increased the destruction of the vitamine only 1.2. In the words of H. C. Sherman, "the velocity of the heat destruction decreased more rapidly than it would if the reaction followed either the unimolecular reaction law or the square root rule of Schutz. Empirically the percentage destroyed was found to vary as the fourth root of the time of heating. . . . The low temperature coefficients and the difference in rate of destruction within and without the plant cells suggest that the reaction involved in the heat destruction of the vitamin is of the heterogenous type."

Again, vitamine C was found to be less susceptible to the action of heat in an acid medium than in a neutral solution, while in the presence of an alkaline reaction destruction of the vitamine was much more pronounced. Thus Sherman and his collaborators found that when about half the natural acidity of tomato juice was neutralized, destruction of the vitamine by one hour's boiling was increased from 50 per cent to 58 per cent. The susceptibility of the vitamine to alkalinity even at low temperatures, as pointed out by Hess and Unger, as well as by other observers, is quite marked.

Further, the experiments of R. A. Dutcher, H. M. Harshaw and J. S. Hall, 1921, *The Effect of Heat and Oxidation upon the Antiscorbutic Vitamin, Journal of Biological Chemistry,* as well as the experiments of Ellis, Steenbock and Hart, 1921, and others, have emphasized

the susceptibility of vitamine C to oxidation, even aeration at a temperature of 100° C. causing rapid destruction of the vitamine. From facts such as these, gathered in many laboratories, it became apparent that in the cooking and canning of foods containing the antiscorbutic vitamine where as much of the latter as possible is to be preserved, the employment of a high temperature for a short time is preferable to the use of a lower temperature for a longer period. With due regard to temperature, reaction, period of time, hydrogen-ion concentration and oxidation, the drying and aging of foods can be accomplished without great loss of the antiscorbutic vitamine.

Many experimental results have been reported by various investigators bearing on the relative antiscorbutic properties of fresh and desiccated fruits and vegetables, among which the work of M. H. Givens and collaborators may be cited: *The Antiscorbutic Property of Desiccated and Cooked Vegetables,* with B. Cohen, 1918; *An Experimental Study of Raw and Dried Tomatoes,* with H. B. McClugage, 1919; *An Experimental Study of Raw and Dried Potatoes,* with H. B. McClugage, 1920; *The Antiscorbutic Property of Some Desiccated Fruit Juices,* with I. G. Macy, 1921, all published in the *Journal of Biological Chemistry.*

Employing young guinea pigs as subjects and a basal ration which produced scurvy, Givens and McClugage found that while the addition of 10 grams of raw white potato to the daily ration protected from scurvy for practically four months, cooking the potatoes in water at 100° C. for one hour destroyed such an amount of the vitamine that even 15 grams of the boiled potato daily

were not sufficient to check the disease. If, on the other hand, potatoes were boiled for fifteen minutes there was only a slight reduction in potency. When potatoes were dried the antiscorbutic power of the product was found to be dependent upon the degree of heat employed. Dried at 35°-40° C. fatal results from scurvy were delayed in some degree by an amount of the dried product equal to 10 grams of the fresh potato, while if dried at 70°-80° C. a like dosage of the dried product was not always able to protect from a fatal result. When dried at 100° C. for one hour the antiscorbutic potency of the potato was destroyed.

The content of vitamine C in potatoes is considerably less than that of many fruits. Thus, Hess on the basis of his experiments estimated that cooked potatoes weight for weight contain only one-fifth to one-tenth the amount present in oranges, lemons and tomatoes, while the amount in apples and bananas is approximately equal to that of the potato. H. B. Lewis in the *Journal of Biological Chemistry,* 1919, reported that with an adequate basal diet the addition of 10-15 grams of banana per day was quite adequate to protect a guinea pig from scurvy. Hess and Unger have pointed out that there is a wide difference in the protective power against scurvy of *old* and *young* carrots, especially when cooked. Thus, they found that while a guinea pig could be protected from scurvy by 35 grams of raw, old carrots, if the carrots were cooked for three-quarters of an hour their addition to the dietary was without effect; on the other hand, 25 grams of fresh young carrots even when cooked a like period of time afforded complete protection from scurvy. Again, the

observed that while young fresh carrots quickly dried were strongly antiscorbutic, carrots grown old before drying had lost, in large measure at least, the power to protect from scurvy. Doubtless, both vegetables and fruits vary greatly in the amount of antiscorbutic vitamine they contain, dependent upon age or maturity and freshness.

The importance of the tomato as an antiscorbutic has been emphasized by Hess and many others. Thus, Givens and McClugage observed that 1 gram of dried tomato —not heated above 40° C.—was sufficient when fed daily to protect a guinea pig from scurvy, while Sherman and his collaborators found that canned tomato juice, when fed to guinea pigs in doses of 3 cc. per day was quite adequate for complete protection. It is also to be noted that in the normally acid juice of the tomato the vitamine was found to be very resistant to heat, boiling the juice for four hours even, destroying only about two-thirds of the vitamine (Sherman).

Animal tissues as a class have been found to be exceedingly poor in vitamine C. R. A. Dutcher previously referred to, head of the department of agricultural and biological chemistry at Pennsylvania State College since 1921, and who has conducted many vitamine studies, reported in 1920 on *The Antiscorbutic Properties of Raw Beef*, with E. M. Pierson and A. Biester in the *Journal of Biological Chemistry*, finding that raw, lean beef was without any noticeable antiscorbutic action on guinea pigs. H. T. Parsons, in a paper entitled *The Antiscorbutic Content of Certain Body Tissues of the Rat*, 1920, *Journal of Biological Chemistry*, stated that the livers of rats long fed on a diet productive of scurvy still showed the

presence of vitamine C, sufficient in amount to demonstrate antiscorbutic action when fed to guinea pigs. Blood, as Hess and others have shown, contains some vitamine C, probably more than is present in muscle, but the amount is not large. There is apparently only a limited power of storage of the antiscorbutic vitamine in the animal body, as Hess and others have pointed out, certainly not equal to the storage of vitamine A. Thus, if guinea pigs are fed liberally with antiscorbutic food for some time they will succumb to a scorbutic diet nearly if not quite as soon as animals fed merely upon a maintenance diet.

Milk has been suggested by Hess as comparable with blood in the amount of antiscorbutic vitamine present, but as many observers have shown, variations in the amount of vitamine present in milk are to be expected, such, for example, as may arise in connection with the character of the food fed to the cows, and subsequent treatment of the milk. Studies of experimental scurvy in guinea pigs by Hart, Steenbock and Smith, 1919, led to the conclusion that an infant, assuming it needs five times the amount of vitamine C required by a guinea pig, will be amply provided with the vitamine in question by one pint of fresh milk daily, in harmony with the findings of other investigators.

In experiments by R. A. Dutcher, C. H. Eckles, C. D Dahle, S. W. Mead and O. G. Schaefer, on *The Influ ence of the Diet of the Cow upon the Nutritive an Antiscorbutic Properties of Cows' Milk,* 1920, publishe in the *Journal of Biological Chemistry,* it was found th on summer feed, when the food might be assumed be rich in vitamine C, 20 cc. of the milk were more th

equal in antiscorbutic potency to three times the quantity of milk from the same cows on winter feed. Similarly, Hart, Steenbock and Ellis, 1920, studying the effect of different feeds upon the antiscorbutic value of cows' milk found that with a ration composed largely of dried grains and hay the addition of as much as 75 cc. of the milk to the basal ration of a guinea pig was required daily to bring about full protection from scurvy.

Again, experiments from various laboratories have indicated that not only is there variation in the antiscorbutic properties of milk dependent upon the character of the food but changes occur in the aging of milk and especially in heating. Thus, Hart, Steenbock and Smith, 1919, found that when milk was heated at 120° C. for four minutes there was a marked loss in antiscorbutic power as measured by the protective effect on guinea pigs. Further, commercial evaporated milk and milk powders were likewise found to have lost some of the antiscorbutic potency originally present in the fresh milk. Sherman, in 1922, came to the conclusion that on an average there is probably a loss of approximately one-half the antiscorbutic power of milk in the methods of drying ordinarily employed. Condensed milk, on the other hand, as Hess, 1921, and others have found, owing to the preservative action of the sugar present retains the antiscorbutic power of the original milk largely unimpaired.

Dietary deficiencies have long been suspected as being responsible, at least in some degree, for abnormalities of the generative functions. In their numerous growth experiments on rats with varied dietaries, Osborne and Mendel noted that frequently there was failure to breed

although the dietary was quite adequate for normal growth and general well-being. In 1922 Herbert M. Evans of the University of California, with K. S. Bishop, reported results of great significance bearing on this subject: *On an Invariable and Characteristic Disturbance of Reproductive Function in Animals Reared on a Diet Poor in Fat-soluble Vitamin A,* published in the *Anatomical Record; On the Existence of a Hitherto Unrecognized Dietary Factor Essential for Reproduction, American Journal of Physiology.* According to the data reported by these investigators, rats fed on a diet composed of casein, starch, lard, inorganic salts, with yeast and vitamine A, grew normally but were generally sterile.

As Evans and Bishop stated, "Natural foodstuffs contain a substance X which prevents such sterility or which cures the disorder occasioned by the purified dietary régime. We have thus been able to witness a comparatively sudden restoration of fertility to animals of proven sterility, and whose controls continued sterile, by the administration of fresh green leaves of lettuce. Even the dried leaves of alfalfa appear to possess a similar potency. . . . The beneficial dietary factor can not be identical with the antiscorbutic vitamine C, for curative effects have been secured when ground whole wheat was added to our purified rations."

The following year, 1923, Barnett Sure, professor of agricultural chemistry at the University of Arkansas, reported the results of experiments under the general title *Dietary Requirements for Reproduction* in the *Journal of Biological Chemistry; I. The Nutritive Value of Milk Proteins from the Standpoint of Reproduction, II. The*

*Existence of a Specific Vitamin for Reproduction.* From the results of his many experiments Sure concluded that "lack of fertility or significant success in rearing of young on milk diets must be attributed to a dietary factor other than protein, the fat-soluble A vitamin, the antirachitic vitamin, or the water-soluble B vitamin." His later experimental data led to the conviction that there is a specific vitamine, distinct from the antixerophthalmic, antirachitic, antiberi-beri and antiscorbutic vitamines that is essential for reproduction, and for this new dietary factor he proposed the term "E." This hitherto unrecognized vitamine he found to be present in Georgia velvet bean meal, polished rice, yellow corn and rolled oats.

The experiments of Mattill and Clayton, conducted at the University of Rochester, Department of Vital Economics, 1926, already referred to in Chapter IX, confirmed the view that vitamine E is a necessary factor in the establishment of fertility. They also observed that yeast is not a source of this vitamine, but that wheat germ and wheat germ oil apparently contain relatively large amounts of the vitamine, since these proved especially potent as curative agents in the treatment of *sterility*.

Pellagra, known for a long period as a disease that could be prevented or cured by a suitable diet, has been the subject of much study during recent years, notably by Joseph Goldberger, who from 1899 until his death was connected with the United States Public Health Service. Some of his more important contributions are the following: *The Cause and Prevention of Pellagra,* 1914; *The Transmissibility of Pellagra; Experimental*

*Attempts at Transmission to the Human Subject,* 1916; *The Experimental Production of Pellagra in Human Subjects by Means of Diet,* with G. A. Wheeler and M. X. Sullivan, 1920; *A Study of the Relation of Diet to Pellagra Incidence in Seven Textile Mill Communities in South Carolina in 1916,* with G. A. Wheeler and E. Sydenstricker, 1920; all published in *Public Health Reports,* Washington; *A Study of the Diet of Non-pellagrous and Pellagrous Households,* with G. A. Wheeler and E. Sydenstricker, 1918, *Journal American Medical Association.* Reference should also be made to two later papers by Goldberger and his associates in the *Public Health Reports,* 1926 and 1927, which contain the results of experiments with butter fat and with carrots in the treatment of so-called black tongue in dogs.

Goldberger's studies made it evident that pellagra was not a transmissible disease, but was associated with narrow dietaries containing excessive amounts of degerminated cereals and vegetables. In one experiment with a group of eleven volunteers from a convict camp in Mississippi, Goldberger and his associates found that on a faulty diet composed of highly milled wheat flour, polished rice, degerminated maize, starch, sugar, sweet potatoes, turnips and turnip greens, cabbage, collards, pork fat, molasses and coffee, six of the eleven subjects developed symptoms of pellagra within six months. It is to be noted that only a very small fraction of the energy value of this ration was derived from fresh vegetables and that aside from molasses and fat pork, the bulk of the food was composed of degerminated cereals and products therefrom.

Eventually, 1926, Goldberger's experimental results and observations led him to believe in the existence of a specific vitamine, which he termed "P-P," *i.e.*, pellagra-preventive vitamine. The addition of milk and of fresh meats in fairly large proportion to the diet of pellagrins proved very beneficial. Yeast was likewise effective, but Goldberger held that the active agent was not the anti-neuritic vitamine B, but a specific substance which he named as above.

Edward B. Vedder, of the United States Army Medical Service, from his observations reported in the *Archives of Internal Medicine*, 1916, under the title *Dietary Deficiency as the Etiological Factor in Pellagra*, emphasized the similarity between pellagra and such deficiency diseases as beri-beri and scurvy, holding the view that pellagra was a true deficiency disease induced by the too exclusive consumption of wheat flour, corn meal and other cereals, together with salted meats and canned goods, obviously with a deficiency of vitamines. Vedder further laid emphasis on the similarity of the gastro-intestinal lesions in pellagra, beri-beri and scurvy, especially the inflammation of the mucous membranes of the stomach and duodenum, diarrhea, enteritis, ulceration of the intestines and hemorrhage into the mucous membranes.

Carl Voegtlin, professor of pharmacology, United States Public Health Service since 1913, who has made many studies of vitamines and deficiency diseases, conducted at the Pellagra Hospital in Spartanburg, S. C., experiments designed to explain the influence of diet on the course of pellagra. Like Goldberger and others he found a causal relationship between the disease and a restricted,

narrow dietary; the addition of one liter of milk, about four eggs and 100 grams of fresh beef to the daily diet bringing about a speedy improvement, and in many cases complete recovery.

In an attempt to discover the reason for the prophylactic value of these animal foods, patients having well-marked attacks of pellagra were placed on a restricted vegetable diet and when their condition was stationary or even growing worse, a fat-free alcoholic extract of yeast, rice polishings, ox liver, or thymus gland, was administered daily. The yeast and rice extracts even when given in large amounts Voegtlin found were without effect on the course of the disease, which he interpreted as indicating that the defect of a pellagrous diet cannot be attributed simply to a lack of antineuritic vitamine.

The liver preparations on the other hand, presumably containing both the fat-soluble and the antineuritic vitamines, led to an improvement in the condition of the pellagrins "apparently comparable to that produced by the consumption of a diet containing a considerable amount of milk, eggs and meat." As Voegtlin wrote in 1920,[4] "The evidence so far available, therefore indicates that the dietary defect presumably responsible for pellagra is distinctly different from, and probably more complex than, the one causing human beriberi." Further, "it is more likely that the pellagrous syndrome is caused by a *combination of the deficiencies in some of the well-recognized food factors*, a hypothesis which would account, first, for the resemblance between the symp-

[4] *The Harvey Society Lectures*, 1919-20, "Recent Work on Pellagra."

tomatology and histopathology of scurvy, beriberi and pellagra, and, second, for the great individual variation in the symptom complex observed in different patients."

In 1917 Chittenden and Underhill in a paper previously cited called attention to the production in dogs of a pathological condition closely resembling human pellagra induced by a diet composed largely of boiled peas, together with cracker meal and cottonseed oil. After a time on such a ration the animals developed inflammation of the mouth with more or less sloughing of the mucosa, and what was particularly noticeable, a peculiar and characteristic appearance due to the inner surface of the cheeks and lips and the edges of the tongue being covered with a mass of pustules. There was also a bloody diarrhea.

In 1922, G. A. Wheeler, J. Goldberger and M. R. Blackstock in *Public Health Reports*, Washington, called attention "to the striking similarity and probable identity of Chittenden and Underhill's pellagra-like syndrome in dogs and the condition known to American veterinarians as 'black tongue,' " to which they added the statement, there is "the possibility, if not the probability, that black tongue in dogs may prove to be the analogue of pellagra in man."

In 1927, Underhill and Mendel reported in the *American Journal of Physiology* additional results bearing on this subject under the title, *A Dietary Deficiency Canine Disease—Further Experiments on the Diseased Condition in Dogs Described as Pellagra-like by Chittenden and Underhill and Possibly Related to So-called Black Tongue.* As to the exact nature of the deficiency responsible for this disease several possibilities were noted, *viz.*, the char-

acter of the protein in the diet, its content of amino-acids, insufficiency of fat-soluble vitamine A, inadequate amount of water-soluble B, a deficiency in calcium and phosphorus and likewise of nitrogen.

By experiments carefully designed to determine these points it was shown that modification of the Chittenden-Underhill diet by the addition of considerable meat and bone ash with its large proportion of calcium and phosphorus did not protect the dogs, nor were the characteristic untoward symptoms modified. Again, replacement of the peas in the diet by lean raw meat failed to maintain the dogs and they died with the typical symptoms. If, however, fresh pigs' liver was introduced into the diet the incidence of the disease was prevented, while the addition of butter fat was found to be most effective, this evidently containing the protective factor in relatively large measure.

The potency of this protective factor was emphasized by the results of a series of experiments in which Underhill and Mendel demonstrated the rôle of butter fat as a curative agent after the disease had been produced by the C-U diet. Thus, it was found that dogs suffering from this deficiency disease could be quickly restored to a normal condition and maintained in health for prolonged periods by simply feeding butter fat. Suggestive is the fact that cod liver oil, well known to contain the fat-soluble vitamine A, did not possess this power, from which it would follow that this vitamine is not the responsible factor in the protective and curative action of butter fat. Further, the active agent in butter fat was found to be quite labile, as was indicated by its diminished potency

after maintenance in cold storage for a period of 9-11 months.

Especially suggestive was the fact brought out by these experiments that the potency of butter fat was associated with the color of the butter; with less color there was less potency. Even more pronounced curative effects were obtained with boiled unpeeled carrots, which when added to the diet of dogs were much more effective than butter fat, both in bringing about a cure and in maintaining the animals in good condition without any other addition to the C-U diet. It was also found that crystallized carotin, even in quite small doses, was potent in curing the diseased condition. Since egg yolk also showed curative action it seemed justifiable to assume that in all probability a carotinoid or some closely related substance, such as the xanthophyll of egg yolk (carotin dioxide) is the responsible agent in protecting against the disease and in alleviating the symptoms when the disease is once acquired.

# CHAPTER XII

Internal secretions—Epinephrine and adrenaline, investigations of John J. Abel, Jokichi Takamine, Thomas B. Aldrich—Bufagin—Thyroid gland and thyroxine, work of Reid Hunt, Fred C. Koch, Edward C. Kendall and others—Insulin, studies by Abel and collaborators—Parathyroid, parathyroid tetany, relation of calcium to, William G. MacCallum and Carl Voegtlin, Carl A. Binger, Isidor Greenwald, E. P. Clark and T. W. Scott—Pituitary body—Tethelin of Robertson—Studies of anterior hypophysis by Herbert M. Evans—Studies of posterior hypophysis by Abel and collaborators—Physiological relationship of the individual hormones.

In the winter of 1894, while in London, I saw much of Sir Edward Schäfer, at that time professor of physiology at University College. He was occupied with experimental work on the adrenal gland with G. Oliver, his assistant in the laboratory being Benjamin Moore, who for a brief period, 1898-1900, served as professor of physiology in the Yale Medical School, later becoming the professor of biochemistry at Liverpool University, and from 1920 to his death in 1922 holding the chair of biochemistry at the University of Oxford. It is to be remembered that Oliver and Schäfer were the first to test the effect of extracts of the adrenals on arterial pressure and it was their work which aroused the interest of chemists.

One day Schäfer said to me, "Come to the laboratory tomorrow, I am going to try an experiment to see if the active principle of the adrenal is soluble in alcohol." Ac-

cordingly the next day I witnessed an experiment, the result of which left no doubt that the potent factor in raising blood pressure could be readily dissolved in alcohol, for after injection of a few minims of the solution (from the adrenal of a cat) into a large dog the writing style of the kymograph began to climb swiftly up until in a few seconds it was vibrating in the air far above the roll of recording paper. Schäfer turned to me and said, "What an active substance it must be. Do you suppose we shall ever be able to isolate it and determine its chemical nature?" This question was answered in the affirmative later on largely through the efforts of biochemists in America, notably John J. Abel, Jokichi Takamine and Thomas B. Aldrich.

John J. Abel, a graduate of Michigan University in 1883, pursued the study of chemistry and medicine at various European universities from 1884 to 1891, becoming in 1893 professor of pharmacology at The Johns Hopkins University. While his chair was that of pharmacology, Abel's activities and accomplishments in various branches of physiological chemistry justly entitle him to high rank among the physiological chemists of America, and during the earlier years of his work at Baltimore he was the responsible leader there in this department of study. Through his influence physiological chemistry became a vital part of the medical curriculum at Johns Hopkins and under his guidance were trained many men who became successful workers in this branch of biological study. Eventually, distinct chairs of physiological chemistry were established at the university, filled by men whose work has already been referred to.

To students of biology in general and of physiology in particular, the organs of internal secretion have during the past thirty years or more come to occupy a position of special importance. Through the combined efforts of physiologists and chemists there has been revealed a method, quite distinct from the action of the nervous system, by means of which communication between individual organs is maintained and harmony of functional activity secured. The method, as is well understood, depends upon the existence of definite chemical substances known as hormones, sometimes termed chemical messengers, the products of various secreting organs. Among these hormones, the blood-pressure-raising constituent of the adrenals was the first to be separated and its chemical nature determined.

Abel, in 1897, with Albert C. Crawford, published his first paper under the title, *On the Blood-pressure-raising Principle of the Adrenals.* This paper was soon followed by others: *Further Observations on the Chemical Structure of the Active Principle of the Suprarenal Capsules,* 1898; *Further Observations on Epinephrine,* 1901; *On the Behavior of Epinephrine to Fehling's Solution and Other Characteristics of This Substance,* 1901, all in *The Johns Hopkins Hospital Bulletin; Über den blutdruckerregenden Bestandtheil der Nebenniere das Epinephrin,* 1899, *Zeitschrift für physiologische Chemie; On Epinephrine and its Compounds with Especial Reference to Epinephrine Hydrate,* 1903, *American Journal of Pharmacy; On the Decomposition Products of Epinephrin Hydrate,* with R. deM. Taveau, 1905, *Journal of Biological Chemistry.*

Abel was at once successful in separating the active principle from aqueous extracts of the gland by treatment with benzoyl chloride and sodium hydrate. By saponification of the benzoyl compound with dilute sulfuric acid in an autoclave, what was thought to be the free base was liberated, from which was prepared a considerable number of salts and derivatives all possessed of a high degree of physiological activity. The elementary composition of the free base, on the basis of various analyses, was represented by the formula $C_{17}H_{15}NO_4$. To this base Abel gave the name of epinephrine.

Unfortunately, as Abel himself eventually ascertained (1901), his epinephrine in the process of saponification had retained a single benzoyl radical, "in consequence of the singular tenacity with which this lone radical defied all but destructive methods for its removal from the original benzoyl compound." It thus became evident that it was in reality a benzoyl compound of the active principle that he had been working with and not the free base. Removal of this retained radical from his "former series of compounds and substitution of the displaced hydrogen atom led to the formula $C_{10}H_{11}NO_3$ as an adequate empirical expression for epinephrin with alkaloidal properties."

Naturally, the pioneer work of Abel quickly drew other workers into the field, and in 1901 Takamine from his research laboratory in New York reported in the *American Journal of Pharmacy* the results of his studies under the title, *Adrenalin, the Active Principle of the Suprarenal Glands and its Mode of Preparation.* The method employed was very simple, consisting essentially of direct

precipitation by ammonia from a sufficiently concentrated extract of the gland. His preparation, which he named adrenaline, was "a light white, microcrystalline substance," possessed of great potency, intravenous injection of the adrenaline showing it to be a thousand times stronger than the fresh glands, "a fraction of one drop of aqueous solution of adrenalin or its salt in strength of 1:50,000 blanches the normal conjuctiva within one minute." Takamine adopted the expression $C_{10}H_{15}NO_3$ as "the probable empirical formula" for adrenaline and patenting his process in this country the substance was manufactured and widely used for its great activity in raising the blood pressure.

At essentially the same date and quite independently, Thomas B. Aldrich had obtained from the suprarenal glands a semi-crystalline substance apparently identical with Takamine's product and in 1901 he published, in the *American Journal of Physiology, A Preliminary Report on the Active Principle of the Suprarenal Gland,* from the Biological Department of the Scientific Laboratory of Parke, Davis and Company of Detroit. A later and fuller account was reported by Aldrich in the *Journal of the American Chemical Society,* 1905.

From Aldrich's work it became clear that, when thoroughly purified, adrenaline had the empirical formula of $C_9H_{13}NO_3$. Observations by Abel and others indicated the presence in the epinephrine or adrenaline molecule of a pyrocatechin-like residue and Abel, from his preliminary work, was inclined to the view that the active principle of the suprarenals must contain a residue or nucleus $C_6H_3(OH)_2$ or $C_6H_2(OH)_3$. In due time it became ap-

parent that adrenaline was in all probability a methyl-amino derivative of pyrocatechol. The work of several European investigators has not only verified the formula established by Aldrich but eventually showed that the active principle of the suprarenal glands is dihydroxy-methyl-amino-ethylol-benzene.

$$C_6H_3(OH)_2 \cdot CHOH \cdot CH_2 \cdot NH \cdot CH_3$$

Since there are two asymmetrical carbon atoms in the molecule it follows that there are two optical isomers. In 1907 Stolz prepared the racemic mixture synthetically and observed that its physiological action was rather more than half that of the natural form (the *l*-isomer) present in the suprarenal gland. Later, the levo-rotary form was also synthesized.

The discovery by Abel in 1911 of two crystalline principles in the secretion from the parotid glands of a tropical toad revealed the fact that adrenaline, hitherto found only in the suprarenal glands and in homologous chromaphil structures, is also present in an animal secretion. Abel and his collaborator, David I. Macht, reported the results of their investigation in the *Journal of Pharmacology and Experimental Therapeutics,* under the title *Two Crystalline Pharmacological Agents Obtained from the Tropical Toad, Bufo agua,* 1911. The milky secretion from the parotid gland of this poisonous toad when diluted was found to yield the characteristic green color of the pyrocatechol reaction with ferric chloride. This led to a chemical study of the secretion with identification of adrenaline by chemical analysis and by physiological tests.

Remarkable was the amount present in the venom; in one analysis 5.42 grams of the secretion yielded 0.243 gram of crystalline adrenaline, equal to 4.48 per cent. On the assumption that the method of separation could not yield more than two-thirds of the amount actually present it was estimated that the fresh secretion must contain approximately 7 per cent of adrenaline, a striking contrast to the 0.3 per cent estimated by Abel as present in the suprarenal glands of beeves.

The second crystalline principle proved to be non-nitrogenous with an elementary composition and molecular weight represented by the formula $C_{18}H_{24}O_4$. It had a melting point of 217°-218° C., was dextro-rotary (+11°) and slightly soluble in water. Treatment with bromine showed that it does not contain an unsaturated carbon linkage of cholesterol. It was named ***bufagin***. Its physiological properties indicated that bufagin is an active member of the digitalis series, having a marked action on the heart, cardio-inhibitory center and on the musculature of the blood vessels, which fact, as the authors state, would explain in large degree the efficacy of the venom as an arrow poison, although the adrenaline may be an important auxiliary.

The discovery by Baumann of Freiburg in 1895 of the presence of iodine in the thyroid gland, followed by the separation of a substance containing 10 per cent of iodine to which Baumann gave the name of thyroiodin, led to the recognition of a causal relation between this iodine complex and such diseases as goiter, cretinism, myxedema and exophthalmic goiter. In other words, there was the suggestion that in the thyroid gland there is produced a

hormone containing iodine as a part of the molecule, the exact nature of which, however, was far from being understood. That there was a physiologically active principle in the thyroid was perfectly clear, but whether this principle was a large complex such as a thyroiodalbumin, a thyreoglobulin, or a smaller complex such as thyroiodin or a still smaller molecule with higher content of iodine remained for some time an uncertainty.

This occurrence of iodine in the animal body in itself aroused great interest and its possible physiological action in some combined form led to wide-spread studies, chemical, physiological and medical in nature, in which both the desiccated gland and compounds prepared therefrom were employed. The curative action of the dried gland when fed to patients suffering from cretinism, goiter and myxedema was most pronounced and excited additional interest in the character of the active hormone. Methods were worked out by various investigators for testing the physiological activity of thyroid substance based on changes in blood pressure, on increase of the irritability of the depressor nerve, on changes in nitrogen metabolism and on the curative effects in cretinism.

One method much used in America was based upon the protective action of thyroid on the poisonous effects of acetonitrile on mice, devised by Reid Hunt of the United States Public Health Service, since 1913 professor of pharmacology at Harvard University, and published, 1905, in the *Journal of Biological Chemistry* under the title *Influence of Thyroid Feeding upon Poisoning with Acetonitrile.* Mention should also be made of the studies

by Hunt and A. Seidell, 1908-1910, on the relation of thyroid activity to iodine content.

Under the title *On the Nature of the Iodine-containing Complex in Thyreoglobulin,* Fred C. Koch of the University of Chicago showed by the use of appropriate physiological tests that thyreoglobulin possessed the full activity per unit of iodine when compared with the dried thyroid from which it was prepared. As he stated, however, it was not to be assumed that the protective action was due to iodine itself but rather to some specific iodine-containing complex in the thyreoglobulin.

In the Proceedings of the American Society of Biological Chemists, December, 1916, Edward C. Kendall, already referred to in Chapter VIII, reported the separation of the effective hormone of the thyroid gland, a crystalline substance containing 65 per cent of iodine, to which he gave the name of *thyroxine.* This crystalline hormone Kendall had prepared in 1914 and thereafter there appeared from time to time various papers from the section of biochemistry of the Mayo Foundation giving the results of his studies of this interesting substance. The following may be cited: *Isolation of the Iodine Compound which Occurs in the Thyroid,* 1919; *The Chemical Identification of Thyroxin,* with A. E. Osterberg, 1919; both published in the *Journal of Biological Chemistry;* also *The Thyroid Hormone,* in the collected papers of the Mayo Clinic, 1916.

In his early work Kendall found that primary cleavage of the thyroid proteins, especially by alkaline hydrolysis, resulted in acid-insoluble A and acid-soluble B constituents, without breaking off iodine from its organic com-

bination. Separation of the iodine compound from A was accomplished by solution in dilute sodium hydroxide, precipitation with barium hydroxide aided by heat, and addition of acid to the filtrate containing the soluble barium compound, after removal of the barium by sodium sulphate. This final precipitate by acid on analysis was found to contain 15 per cent of iodine in contrast to the 5 per cent present in the material started with. By successive treatments with alkali and acid the product was brought step by step to a higher degree of purity with progressive iodine content, until finally on removal of the last traces of impurities by solution in alkaline alcohol and precipitation with acetic acid, the free base was obtained as microscopic crystals with 65 per cent of iodine. The hormone could also be precipitated in salt form, the salt of sulfuric acid containing 60 per cent of iodine. The molecular weight determination led to 586, while ultimate analysis pointed to the empirical formula of $C_{11}H_{10}O_3NI_3$ for the free base.

In physiological activity thyroxine proved to be highly potent, 0.125 to 0.250 milligram daily being sufficient for cretinism, while the maximal quantity tolerated by human beings was 2 milligrams daily. Excessive doses given to animals produced symptoms of hyperthyroidism followed by death. As stated by Kendall and Osterberg, thyroxine "is odorless and colorless and is not easily affected by oxidation or reduction, its most important chemical and physical properties are concerned with the acidic and basic groups within the molecule." It is a weak acid, although it manifests basic properties in the presence of mineral acids. Quite remarkable are the varied crystalline

possessing however the highest value in alleviating the symptoms of diabetes in human subjects.

There remained the task of isolating, separating and determining the chemical nature of the true insulin hormone contained in these relatively crude extracts. Toward these ends John J. Abel and his collaborators have contributed much, as the following citations will indicate: *Researches on Insulin, Is Insulin an Unstable Sulphur Compound?* with E. M. K. Geiling, 1925; *Crystalline Insulin,* with Geiling, C. A. Rouiller, F. K. Bell and O. Wintersteiner, 1927, both published in the *Journal of Pharmacology and Experimental Therapeutics; Crystalline Insulin, Proceedings National Academy of Sciences,* 1926.

Starting with commercial insulin from the laboratories of the Eli Lilly Company of Indianapolis, one lot evaluated at approximately 8 rabbit units [1] per milligram, a second lot showing 12 rabbit units to the milligram, Abel and his associates found, in harmony with other workers, that crude insulin was an exceedingly complex mixture made up of many closely related substances. If to an aqueous solution of such commercial products, a little pure pyridine was added a flocculent precipitate began to appear, which on the addition of a sufficient volume of N/6 pyridine solution gave place to complete precipitation. On separation of the precipitate by centrifugalization, dissolving it in water to which a sufficient quantity of N/6 acetic acid was added, and reprecipitating with pyridine the active principle could be separated from the

[1] A unit being the minimal amount, per kilogram, which will cause convulsions or reduce the blood sugar to the convulsive level.

inert material, which remained largely in the pyridine filtrates. By repeating this operation five or six times "from 35 to 40 per cent of material which consists almost entirely of inactive substances is removed."

Further purification was accomplished by treatment of the final pyridine precipitate with 90 per cent phenol, much impurity remaining undissolved, while the active insulin was held in solution and could be separated by the addition of a large volume of water, when it took on the form of a sticky precipitate adhering to the sides of the flask. Still further purification was accomplished by dissolving this cleaner insulin in very dilute acetic acid and precipitating by the addition of a small amount of a saturated solution of sodium chloride, the salt being removed by dissolving the precipitate in water acidulated with acetic acid and precipitation with N/6 pyridine.

While not perhaps a chemically pure product, this proved to be a highly concentrated form of insulin "able to reduce the blood (of rabbits) to the convulsive limit at 40 units or more to the milligram." One of the striking facts brought to light by study of this product was that the content of labile sulfur was directly proportional to the degree of hypoglycemic activity. Or, expressed differently, the higher the amount of "sodium carbonate sulphur" present in a given preparation the greater was its potency.

Eventually a short and simple method was devised by Abel and his collaborators which yielded the hormone as "glistening highly refractive crystals," very uniform in character, suggestive of a high degree of purity. In brief, the method consisted in the addition of brucine acetate—

1 gram of base in 18 cc. of N/6 acetic acid—and a solution of pyridine to an acetic acid solution of the insulin powder, the resulting pyridine precipitate removed by centrifugalization and the filtrate treated with 0.65 per cent aqueous ammonia. On standing, crystals of the hormone separate, usually adherent to the glass walls of the flask. As stated by Abel, "the mixture of acetic acid, brucine and pyridine is, of course, a highly buffered solution, and only when a further great excess of pyridine or, preferably, of 0.65 per cent ammonia is added until the solution attains a pH of 5.55 to 5.65 does the insulin begin to crystallize out."

Crystalline insulin gave on analysis results which would imply an empirical formula of $C_{45}H_{69}O_{14}N_{11}S$ for the dehydrated substance. Of the total sulfur 37.41 per cent was in the labile form. The crystals were levorotary, the degree of rotation depending largely on the concentration and pH of the solution as well as on the nature of the solvent. Striking reactions were given with the biuret test and with Millon's reagent, the Pauly and the ninhydrin reactions also being positive. On the other hand, the tryptophane reaction of Hopkins-Cole was negative, as was also the Sullivan test for free cystine and cysteine. The extreme sensitiveness of pure insulin to alkali is apparent from the fact that when boiled for fifteen minutes with N/10 sodium carbonate its hypoglycemic power is entirely destroyed. As to the potency of crystalline insulin in producing hypoglycemia, Abel found that "amounts as small as 1/100 of a milligram per kilo lowered the blood sugar to about the convulsive level, 0.045 per cent."

The parathyroid glands afford another illustration of the great physiological powers possessed by many glandular structures which until recent years have remained largely unrecognized. When, however, parathyroidectomy was performed on dogs there was revealed the existence of an internal secretion or hormone of great potency, the lack of which gives rise to violent clonic convulsions or tetany. To William G. MacCallum and Carl Voegtlin, 1909, we owe the knowledge that parathyroid tetany is accompanied by a low content of calcium in the blood, implying a causal relationship, while later observers, notably Dragstedt and Sudan, 1926, of the University of Chicago, have shown that parathyroidectomized dogs may be kept free from tetany for long periods by the administration of calcium lactate.

Removal of calcium from the circulation of normal dogs by the intravenous injection of dibasic phosphate was found by Carl A. Binger, 1917-1918, while at The Johns Hopkins Medical School, to produce tetany, the calcium in the blood serum being reduced from 10 milligrams to 6 milligrams. For some years it was thought that a relationship existed between these clonic convulsions and alkalosis, but A. B. Hastings and H. A. Murray, Jr., at the College of Physicians and Surgeons, Columbia University, *Observations on Parathyroidectomized Dogs*, 1921, *Journal of Biological Chemistry*, found that while removal of the parathyroids resulted in a calcium deficiency in the blood serum, there was no disturbance of the acid-base equilibrium; the pH of the plasma remained within normal limits.

In 1923 Isidor Greenwald of the Harriman Research

Laboratory, Roosevelt Hospital, reported in the *Journal of Biological Chemistry*, under the title *Alkalosis, Sodium Poisoning and Tetany*, various experiments, the results of which led him to the belief that no specific connection exists between an increased alkalosis of the blood and the appearance of convulsions. He stated that "Tetany and convulsions are not due to any single cause. Any one of a multitude of disturbances in the equilibrium within certain tissues may be responsible. Convulsions are to be regarded as a sign of approaching or partial disintegration of the neuromuscular apparatus."

Greenwald, however, clearly recognized that some substance produced in or by the parathyroid glands is essential for the maintenance of calcium in the blood plasma at a normal level. To render the story more nearly complete it is necessary to refer to two investigations carried on in the Dominion of Canada by James B. Collip of the University of Alberta under the titles *The Extraction of a Parathyroid Hormone Which Will Prevent or Control Parathyroid Tetany and Which Will Regulate the Level of Blood Calcium; The Effect of a Parathyroid Hormone on Normal Animals*, with E. P. Clark and T. W. Scott, both published in the *Journal of Biological Chemistry*.

The three important findings in the first paper were, first, that an extract prepared from the parathyroid glands of oxen will prevent or control parathyroid tetany in dogs; second, that the active principle in this extract produces its effect by causing the calcium content of the blood serum to be restored within normal limits; third, that an overdose of the extract leads to a condition of hypercalcemia with vomiting, drowsiness verging into

coma, and failing circulation which may prove fatal. With normal dogs it was found that subcutaneous injection of the extract raised the level of calcium in the blood serum, while successive injections were followed by profound hypercalcemia which if long continued proved fatal. The blood in fatal cases showed definite changes of both a physical and chemical nature.

The following year Collip and Clark reported the results of an attempt to separate the pure hormone from the gland. The final product, obviously not a chemically pure substance, was a dry amorphous powder possessed of marked physiological activity, containing 15.5 per cent of nitrogen and some iron and sulfur. It showed the ordinary protein reactions and was free from phosphorus; it was quite soluble in 80 per cent alcohol and readily soluble in water either side of its isoelectric point. At pH 4.8 a very sharp isoelectric precipitation of the active substance took place. While there is as yet no clear understanding of the true chemical nature of this active substance it is perfectly manifest that the parathyroid glands, like so many other glands in the animal body, produce a hormone of specific character upon which its physiological potency depends.

Turning now to the pituitary body or the hypophysis cerebri, evidence accumulated during many years from various sources has revealed the fact that this structure at the base of the brain manufactures or yields one or more internal secretions endowed with marked physiological properties. Extirpation of the pituitary in dogs, as noted by many observers, results in a train of very striking symptoms, with slowing of tissue metabolism, re-

tardation of growth, and inactivity of the reproductive glands, followed it may be by death. Physiologists soon learned, however, the necessity of discriminating between the anterior and posterior lobes of the hypophysis, partly as a result of the apparent differences in histological appearance and partly from growing knowledge regarding physiological behavior.

The anterior lobe came to be associated with growth and in 1916 T. Brailsford Robertson, previously referred to in Chapter IX, in his *Experimental Studies on Growth,* devoted much effort to investigation of the growth principle of the anterior lobe of the hypophysis. He separated from the lobe a substance—or more probably a mixture of substances—to which he gave the name of ***tethelin.*** This he considered to be the growth-controlling principle of the anterior lobe of the hypophysis, and he found that it caused marked retardation of growth of the white mouse during the first ten weeks of the third or adolescent growth cycle, followed by pronounced acceleration of growth. It was quite different in its physiological properties from extracts of the posterior lobe of the hypophysis. As to the chemical nature of tethelin, it was obviously a lipoid substance, containing 1.4 per cent of phosphorus, and nitrogen in the proportion of four atoms for every atom of phosphorus. Robertson considered that two atoms of nitrogen were present in amino groups and one in an imino group. On hydrolysis with barium hydroxide followed by hydrolysis with sulfuric acid it yielded *dl*-inosite.

To Herbert M. Evans of the University of California we owe much regarding our understanding of the function

of the anterior hypophysis, especially by his experiments carried on in 1921-1924, which, as he stated, were directed primarily to showing the relation of the glands of internal secretion to the gonads and especially the ovary. Feeding of the anterior lobe of the hypophysis to growing rats he found was followed by a marked acceleration of growth, leading to an increase of size far beyond the normal. In the words of Evans "The hypophysis giants were, in late stages, twice as heavy as the largest individuals known to us from our own and the published records for this animal species. Studies of these animals showed that they were not merely fat animals but that a true overgrowth was participated in by the skeleton and by most of the viscera."

Besides gigantism, feeding of the anterior hypophysis Evans found produced another effect, *viz.*, impairment or prevention of the growth and maturation of the ova, hence there are at least two distinct functions associated with the anterior lobe of the pituitary, which implies the probable existence of two distinct hormones. In harmony with this view, Evans found that the addition of alcohol to extracts of the anterior lobe, in quantity not to exceed 50 per cent, caused a destruction of the growth effect while the ovulation inhibitory effect remained unimpaired. Apparently, the growth hormone was lost in concentrations of alcohol between 8 and 50 per cent. Again, it was found that "acetic acid added as 0.2 N solution so that its final strength is 0.03 N (with a pH hence between $10^{-4}$ and $10^{-3}$) precipitates most of the proteins and yet preserves both hormones." On the other hand, where the proteins of the extract were coagulated by heat and alcohol,

ovarian and growth effects were lacking in both the precipitate and filtrate.

It is thus clearly recognized that the anterior hypophysis is indispensable for growth to adult stature; an excess of the hypophyseal hormone leading to acromegaly and gigantism, while a lessened amount of the hormone, as Evans has stated, is "the direct cause of an important group of, if not all, endocrine dystrophies." In other words, hypophyseal deficiency and hypophyseal excess are both productive of marked alteration of tissue metabolism, due in ultimate analysis to the presence or absence of two or more distinct chemical agents.

As previously stated, the physiological effects which follow the administration of extracts of the posterior lobe of the hypophysis are quite different from those produced by the anterior lobe, thus implying an internal secretion of a different character. Aqueous or saline extracts of the posterior lobe administered to dogs cause among other effects a great stimulation of plain muscle tissue, contraction of the uterus, constriction of blood vessels and inhibition of the excretion from the kidneys in diabetes insipidus. Obviously, there must be one or more hormones responsible for these effects. On this subject much light has been shed by John J. Abel and his collaborators at The Johns Hopkins University, the successive reports of their work being published in the *Journal of Pharmacology and Experimental Therapeutics*.

In 1919 Abel with Kubota called attention to the presence of histamine ($\beta$-iminazolyl-ethylamine) in the hypophysis cerebri, and at that time drew the conclusion that this substance is "the plain muscle-stimulating and

depressor constituent of the posterior lobe of the pituitary gland." Since, however, histamine occurs in many tissues of the body it could not be looked on as a substance peculiar to the pituitary. Abel and Macht, 1919, showed, however, that there was close parallelism in the action of pituitary extracts and histamine salts on the plain muscle of the uterus of the mouse and of the guinea pig, both tracts of muscle, with small and comparable doses, showing increase of tonus, while with larger doses of histamine and pituitary extract there was paralysis of the muscle tracts.

The following year Abel and Nagayama, working with the posterior lobe or infundibular portion instead of the entire gland, pointed out that infundibular extracts prepared without long exposure to acids contained only a small amount of histamine in contrast to the larger quantities of this amine found in therapeutical preparations of the gland. Even brief treatment of a fresh infundibular extract with 0.5 per cent hydrochloric acid for half an hour on a water-bath completely abolished the blood-pressure raising action of the extract and caused a decided increase in the amount of free histamine. Infundibular extracts which had been treated with acids were found to cause a marked fall in arterial pressure in place of the rise of pressure generally observed with untreated extracts. Further, treatment with acid did not destroy, completely at least, the plain-muscle stimulating power of the extract.

The histamine and histamine-like substance obtained after mild treatment with acids they concluded were not specific constituents of the infundibulum. They were led

to the belief that "the infundibulum contains but one active specific substance or hormone, and that this in its uninjured state is not only a blood-pressure-raising but also a plain-muscle-stimulating substance." As Abel stated later there are plainly two minor substances in the hypophysis, one being histamine and the other a proteose-like body, both having the power to lower blood pressure and both mildly oxytocic. To the presence of these two extraneous substances much of the confusion which has existed in the physiological study of pituitary extracts has apparently been due.

In 1922 Abel and Rouiller by a new method of treatment obtained a preparation of the pressor-oxytocic principle of the infundibulum "equal in oxytocic activity to from 20 to 30 times its weight of the acid phosphate of histamine." They found on intravenous injection a pure pressor vaso-motor response while the actively secreting kidney (rabbit) showed a diminished secretion, or even a complete suppression of urinary flow. They came to the conclusion that these results were "the expression of the manifold physiological properties of one and the same hormone." Their methods of preparation, they believed, produced a complete separation of the pressor-oxytocic substance from the depressor substances.

In 1923, Abel, Rouiller and Geiling reported under the title *Further Investigations on the Oxytocic-pressor-diuretic Principle of the Infundibular Portion of the Pituitary Gland,* additional results regarding the chemical and physiological properties of this apparently single substance. Employing raw material prepared for them by the Research Laboratory of Armour and Company, the

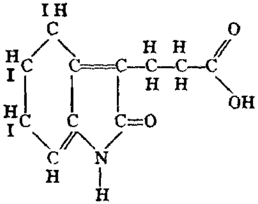
process of extracting the active principle was somewhat as follows: The posterior lobes of the glands were ground to a fine paste with 0.35 per cent hydrochloric acid containing mercuric chloride with ultimate formation of an insoluble "mercuric chloride-protein cake," by which the active principle was adsorbed quantitatively. On disintegration of this cake the mercury was removed by hydrogen sulfide and the active principle precipitated with phosphotungstic acid.

By treatment of this crude precipitate with dilute sodium carbonate and reprecipitation with hydrochloric acid—a process which was repeated several times—the phosphotungstate was purified somewhat, after which the phosphotungstic acid was separated by means of barium hydroxide and the active principle precipitated from the filtrate by the addition of tannic acid and sodium chloride. The tannate precipitate was next converted into a soluble tartrate by treatment with 95 per cent alcohol containing tartaric acid and the salt then precipitated by anhydrous ether.

By various methods of purification, which cannot be detailed here, the "tartrate" was freed from impurities, in large measure at least, so much so that the final product caused "pronounced contractions of the virgin guinea pigs' uterus in a dilution of 1:18,750,000,000"; a fact which testifies at least to the great potency of this oxytocic principle. Further, it is to be noted that the oxytocic value of the most powerful tartrate was 1,000 to 1,250 times that of histamine acid phosphate. The rough outline of the process of separation, as given above, may be taken as showing, whatever the exact chemical nature

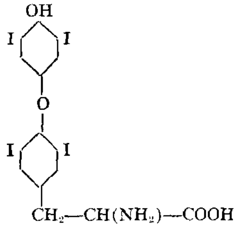
forms of the salts of thyroxine, such as the hydrochloride, the monopotassium salt, the disilver salt, the mono-ammonium, the zinc salt and the acetyl derivative, all of which were thoroughly studied.

In 1915 Kendall suggested, as one of the results of his investigations, that the organic nucleus of thyroxine is indole, that its chemical structure is related to that of tryptophane "from which it is probably derived" and he gave to the compound the name "thyro-oxy-indol, which has been abbreviated to thyroxin." The formula assigned by Kendall to thyroxine, on the strength of the properties and reactions of the compound, was "a tetra-hydro derivative of indole, the three atoms of iodine being substituted for three of hydrogen on the reduced benzene ring."



To Kendall belongs the great credit of having isolated the hormone of the thyroid gland in pure and crystalline form, but unfortunately he was led astray in his conclusions regarding the chemical structure of the molecule. Recent studies of the problem by Harrington and by Harrington and Barger of Great Britain, 1927, who eventually succeeded in preparing synthetically the race-

mic form of the hormone, have proved quite conclusively that the true formula is $C_{15}H_{11}O_4NI_4$ and that its constitution is represented by the following:



*β — [3, 5-diiodo — 4 — (3′, 5′-diiodo — 4′ — hydroxy-phenoxy) phenyl] — a — aminopropionic acid.*

The synthetic compound was shown to be identical in every respect with the natural hormone as obtained by Kendall.

Dating from the well-known discovery by v. Mering and Minkowski, 1889-1891, that extirpation of the pancreas in dogs results in an abnormal condition similar to that found in human diabetes, workers in all countries have been searching for the specific hormone or other active agent in the pancreas responsible for the normal burning of sugar in the body.

The crucial work carried on in the laboratories of the University of Toronto, 1922, *viz.*, by Macleod, Banting, Best and Collip led to the discovery of insulin—an antidiabetic hormone—an internal secretion from the islands of Langerhans. This, to be sure, was in no sense a chemically pure product but rather a therapeutical preparation,

of this active principle or hormone, that it can be carried along step by step to increasing potency and presumably to increasing chemical purity, *i.e.*, that it must be in all probability a chemical substance of definite entity.

At the same time it would appear that precipitation of the active principle depends upon its adsorption by the various flocculent precipitates with their enormous surface as in the use of mercuric chloride, which brings about adsorption of the hormone through formation of the mercuric chloride-protein cake with its large adsorbing surface of precipitated proteins, and later adsorption by the colloidal mercuric sulfide and still later by tannic acid in the presence of sodium chloride, the active principle being liberated from these precipitates through simple alteration of the surface conditions. Again, it was found that the precipitation attending the formation of the above-mentioned mercuric chloride-protein cake effected "a clean-cut separation of substances that act on the blood pressure," these remaining in the acid filtrate.

Convincing proof that the purest tartrate salt obtained represents a single definite hormone of chemical purity is wanting, yet the fact that this tartrate will produce contractions of the uterine muscle in a dilution of more than eighteen billion times and that it retains this potency after various precipitations would imply a degree of strength and stability that might well be attributed to a pure hormone. In any event there is evidence here of the presence in the posterior lobe of the pituitary of a hormone-like substance endowed with marked and powerful properties, which justify the use of the term of oxytocic-pressor-antidiuretic principle. As to its exact chemical

nature there is little definite knowledge and there is lacking chemical evidence of complete freedom from adherent impurities.

The very use of the term chemical messengers as applied to hormones carries with it the suggestion of a means of communication between individual organs, or perhaps better a relationship in function which begets harmony of action and implies an interdependence of more or less physiological significance. The pituitary, the thyroid, the parathyroid, the pancreas, and the suprarenal glands afford good illustrations of this physiological relationship in function. But as Lusk [2] has expressed it, "The subject of the correlation between the various glands of internal secretion is evidently one as replete with opportunities for the play of the imagination as it is for enlightening experimental research." Evans, whose broad study of the glands of internal secretion in general and of the hypophysis in particular gives weight to his views, has stated [3] that "the hypophysis stands in a necessary relationship to normal function of the thyroid, sex glands and adrenal cortical tissue. Any explanation of how these effects are mediated constitutes part of the ill-understood field of chemical dependencies and correlation within the body, ignorance of which cannot properly place in doubt evidence that that correlation exists."

Again, the view is held by some physiologists, especially European, that the activities of certain of the endocrines or hormones, notably insulin, adrenaline and thyroxine are under normal conditions in a state of bal-

[2] "The Science of Nutrition," 1928.

[3] *The Harvey Society Lectures*, 1923-1924.

anced equilibrium one with another, and whenever this balance is interfered with some disturbance of metabolism may result. There is thus suggested a form of automatic adjustment by which necessary functional equilibrium may be maintained.

On this broad subject of physiological relationships between the various internal secretions, American physiologists and physiological chemists in many laboratories have done much work, notably in connection with the study of metabolism in experimental diabetes, while clinicians and hospital workers have likewise made contribution to the general knowledge. It seems not too much to say, however, that complete understanding of the intricacies of function associated with the internal secretions must await the clarifying knowledge of the exact chemical nature of the active hormones, and this physiological chemists and organic chemists will in time supply.

In this very incomplete review of the evolution of physiological chemistry in the United States during the past fifty years, it has been quite impossible to do more than touch lightly upon many phases of work whose importance rightly called for more extended presentation. Further, many lines of experimentation equally deserving of consideration have been left entirely unnoticed, not through lack of appreciation of the results obtained but simply from a determination to keep the book within certain limits of size. That selection was inevitable is quite apparent when it is recalled that the *Journal of Biological Chemistry* alone, representing only one channel of com-

munication, contains eighty-four large volumes filled with the results of American work.

Again, it is to be remembered that this is not a review of the *present-day* knowledge of physiological chemistry, but rather an attempt to show as clearly as possible the development of the science *in this country*. The work in other countries has not been touched upon to any extent, merely here and there a reference to accomplishments where such mention seemed necessary or desirable in order to render a given presentation more intelligible. Further, in order to bring out clearly the character of the development that has taken place in America the past has frequently been stressed rather than the present, since in no other way could an adequate picture of the course of progress be outlined.

Today, it is quite safe to state that in the United States there is hardly a university, worthy of the name, that does not have on its staff one or more competent investigators in physiological chemistry and a well-equipped laboratory with resources adequate for at least some lines of research in this field. Added to these centers of research are the many special laboratories connected with the larger hospitals of the country where investigations of a chemico-physiological character are conducted; the agricultural experiment stations to be found in every state in the Union where work especially on animal nutrition and on phytochemistry are conspicuous features; the many research institutions and foundations with their large endowments, where many biochemists of national reputation are to be found; and lastly the various government bureaus at Washington with their well-equipped labora-

tories and trained workers who have contributed largely to the development of knowledge of a chemico-physiological nature in many directions.

It is only necessary to refer to the Department of Agriculture, with its Bureaus of Plant Industry, Animal Husbandry, Soils, Dairying, Chemistry with its Protein and Nutrition Division, and Home Economics, when there come to mind the names of many physiological chemists who have accomplished work of high character in their respective fields. Still another agency at Washington that has contributed much to chemico-physiological knowledge is the Bureau of the Public Health Service, with the Hygienic Laboratory, where successive workers on biochemical subjects have conducted many investigations that have led to results of great value.

With all the foregoing agencies at work and with a growing appreciation of the importance of this increasing knowledge of the chemico-physiological processes of the animal body, there would seem to be good ground for the belief that the future of physiological chemistry in this country is full of promise, and that what has been accomplished is but the forerunner of more nearly perfect knowledge.

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